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THEME ISSUE

HUMAN IMMUNODEFICIENCY VIRUS—AIDS *Henry S. Sacks, guest editor*

Growth Failure as the First Expression of Malnutrition in Children with Human Immunodeficiency Virus Infection *Vicki B. Peters, Joel R. Rosh, Lisa Mugrditchian, Audrey H. Birnbaum, Keith J. Benkov, David S. Hodes, and Neil S. LeLeiko* 1

Effect of Antiviral Drugs and Hematopoietic Growth Factors on *In Vitro* Erythropoiesis *Rona S. Weinberg, Eileen D. Chusid, Yelena Galperin, JoAnn C. Thomson, Tony Cheung, and Henry S. Sacks* . . 5

Diagnosis and Management of Tuberculosis in Patients with HIV Infection *David N. Rose, Steven Vetter, and Alexandra Gurtman* 14

Passive Immunization for the Treatment of HIV Infection *Jeffrey M. Jacobson* . 22

HIV-Associated Nephropathy *Jonathan Winston and Paul E. Klotman* 27

Cardiac Tamponade in a Patient with AIDS: A Review of Pericardial Disease in Patients with HIV Infection *Lois Estok and Frances Wallach* . 33

DERMATOLOGY NOTES

Risks of HIV Infection *Ron Shelton and Mark Lebwohl* 40

EDITORIAL

Medical Editors Trial Accounting (META) *Sherman Kupfer* 41

ABSTRACTS

Research in Medicine 1997 Mount Sinai School of Medicine 43

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Growth Failure as the First Expression of Malnutrition in Children with Human Immunodeficiency Virus Infection

Vicki B. Peters, M.D.,[1] Joel R. Rosh, M.D.,[2] Lisa Mugrditchian, M.S.R.D.,[2] Audrey H. Birnbaum, M.D.,[2] Keith J. Benkov, M.D.,[2] David S. Hodes, M.D.,[3] and Neal S. LeLeiko, M.D., PhD[2]

Abstract

Background: To define the onset, pattern, and earliest manifestations of malnutrition related to HIV infection.

Methods: A retrospective cross-sectional analysis of changes in weight and growth in a group of 54 children with perinatally acquired HIV infection was conducted. Eight children had asymptomatic HIV infection, 26 had symptomatic infection, and 20 had symptomatic infection and were referred for nutritional support.

Results: We found an early decline in the rate of linear growth with a relative preservation of the weight-for-age. Weight-for-height measurements were preserved until there was advanced HIV-related disease.

Conclusions: This pattern can result in a false impression of adequate nutrition and emphasizes the importance of longitudinal growth data of the child with HIV infection. Evidence of linear growth failure before clinical wasting is apparent is an absolute indication for aggressive nutritional support. **Key Words:** malnutrition, growth failure, human immunodeficiency virus

WEIGHT LOSS AND WASTING in adults and growth failure in children are manifestations of malnutrition. When these symptoms occur in children with human immunodeficiency virus (HIV) infection, they are usually attributed to poor oral intake (1), malabsorption (2), and hypermetabolism caused by intercurrent infection (3), and HIV infection itself (4). Since the earliest reports of HIV infection in children, successful nutritional repletion has been observed (5). The onset of severe malnutrition, however, has invariably preceded nutritional intervention. Kotler et al. (6) examined the impact of malnutrition on survival in adults with acquired immunodeficiency syndrome (AIDS). They concluded that the timing of death from wasting in AIDS was related to the magnitude of body cell mass depletion and not to the specific disease process that caused the depletion. They estimated that at the time of death body weight was 66% of ideal body weight.

We undertook a cross-sectional analysis of changes in weight and growth in a group of children with perinatally acquired HIV infection to define the onset, pattern, and earliest manifestations of malnutrition related to HIV infection.

Methods

We retrospectively reviewed the charts of children with perinatal HIV exposure and con-

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Department of Pediatrics, Divisions of [1]Ambulatory Pediatrics, [2]Pediatric Gastroenterology, and [3]Pediatric Infectious Diseases, The Mount Sinai School of Medicine, New York, NY 10029.

Presented in part at the Society for Pediatric Research Meeting, Washington D.C., 1993. Address correspondence to Neal LeLeiko, M.D., Ph.D., Division of Pediatric Gastroenterology, The Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029.

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firmed HIV infection who were followed up in the Pediatric Infectious Disease Clinic at the Mount Sinai Hospital. Standard anthropometric measurements of height-for-age, weight-for-age, and weight-for-height were recorded for each child at the most recent outpatient clinic visit and at the time of hospital admission. Children were classified by clinical categories according to the criteria of the Centers for Disease Control and Prevention (CDC) (7). Category E designates perinatal HIV exposure in infants younger than 18 months of age who have antibody to HIV but no other evidence of infection. Category N designates asymptomatic HIV infection. Category A, mildly symptomatic infection, includes such conditions as lymphadenopathy and hepatosplenomegaly. Category B, moderately symptomatic infection, includes hematologic complications, single episodes of serious bacterial infections, and lymphocytic interstitial pneumonitis. Category C, severely symptomatic infection, includes AIDS-defining conditions. We created an additional category H that included children with symptomatic HIV infection (Categories A to C) who were referred to the Pediatric Nutrition Support Service during hospitalization for an HIV-related complication. Children in Category H received enteral nutrition, parenteral nutrition, or both.

The mean values of each anthropometric measurement for children in each category were calculated. *Z*-scores, i.e., measurements of the number of standard deviations that exist between a measurement and the reference mean, were calculated for each child's measurements. The *Z*-score was calculated by the formula:

$$Z = \frac{\text{The difference between the measurement and the reference mean}}{\text{The reference standard deviation}}$$

National Center for Health and Statistics (NCHS)-CDC reference heights and weights were used (8). The mean *Z* score of each anthropometric measurement within each patient classification was calculated and used to compare data between the patient classes.

Statistical Analysis. The two-tailed Mann-Whitney *U* test was used to evaluate differences between median values of age and growth parameters among the different groups of children.

Results

The charts of 54 infants and children with confirmed HIV infection and 16 children with HIV perinatal exposure were reviewed (Table 1). Among children with HIV infection, 8 had asymptomatic infection (Category N), 26 had symptomatic infection (Categories A to C), and 20 had symptomatic infection and were referred for nutritional support (Category H). Among children in Category H, 11 (55%) died within 6 months of their referral. None of the children exposed to HIV were subsequently found to have HIV infection at 18 months of age. Mean percentiles and mean *Z*-scores are shown in Tables 2 and 3.

Weight-for-Age. The mean percentile for weight-for-age was virtually normal (48th percentile) for children in Category E, declined in children in Categories N and A to C, but to the same degree, and decreased profoundly in children in Category H.

Height-for-Age. The decline in mean height-for-age percentile correlated with disease progression. Stunting, i.e., weight-for-age below the 5th percentile, was present in children in Category H (*Z*-score = –9.5).

Weight-for-Height. Weight-for-height was preserved at normal values around the 50% percentile in children in Categories N and A to C but was markedly decreased in children in Category H.

As shown in Table 2, there were no statistical differences among children in Category N and A to C regarding the growth parameters. In con-

TABLE 1

Patient Characteristics[a]

HIV disease category	No. children	Mean age (range), months[b]
E	16	7 (3–12)
N	8	25 (2–57)
A–C	26	50 (8–132)
H	20	52 (1–168)

[a] Statistical analyses by two-tailed Mann-Whitney *U* test.

[b] E vs N, E vs A–C, E vs H, $p < 0.005$; N vs A–C, N vs H, A–C vs H, p = NS.

TABLE 2

Mean Percentiles According to HIV Disease Category[a]

	E (n = 16)	N (n = 8)	A–C (n = 26)	H (n = 20)
Weight-for-age[b]	48	30	33	<3
Height-for-age[c]	37	19	20	3
Weight-for-height[d]	60	47	47	10

[a] Statistical analyses by two-tailed Mann-Whitney *U* test.

[b] E vs N, E vs A–C, and N vs A–C, p = NS; E vs H, N vs H, A–C vs H, $p < 0.0003$.

[c] E vs N, N vs A–C, p = NS; E vs A–C, $p = 0.05$; E vs H, N vs H, A–C vs H, $p < 0.006$.

[d] E vs N, E vs A–C, and N vs A–C: p = NS; E vs H; N vs H, A–C vs H: $p < 0.0013$.

trast, there were significant differences when comparing children in Category N and Category A to C with children in Category H for weight, height, and weight-for-height.

Discussion

Wasting, i.e., a decrease in height below the 5th percentile, is the hallmark of the acutely malnourished child (9). It may result from a severe acute illness in a well child or from a series of recurrent illnesses in children with an underlying disease. The well child recoups the nutritional deficits and resumes normal growth. The child with recurrent illnesses does not have the opportunity to recoup nutritional losses, continues to deplete fuel reserves, and fails to grow. Prolonged inadequate nutrition leads to stunting, i.e., height-for-age below the 5th percentile (9). In children with recurrent infection, weight-for-height is low because wasting precedes stunting.

We observed a different pattern in our group of children with HIV infection. Linear growth was affected earlier and more profoundly than body weight. As a result, there was a relative preservation of the weight-for-height for children in Categories N and A to C. This pattern manifested as children who were inconspicuously short and appeared to have appropriate body weight. They did not appear wasted or malnourished. Comparison with age-matched children in Category E allowed us to control for environmental factors affecting our study population. The anthropometric data for this group were different from the data of the infected cohort. The slightly low height-for-age was consistent with the skewed results of the NCHS-CDC data for children younger than 2 years of age (8, 10, 11). These children had otherwise appropriate weight-for-age and weight-for-height. The disturbed growth pattern in children with HIV infection appeared to be a complication of HIV infection. Differences we found between children exposed to HIV infection and age-matched children with HIV infection confirm the differences reported by other investigators (12).

There are two possible mechanisms for the pattern of growth disturbance we observed in our group of children with HIV infection. Centrally mediated, hormonal pathways such as the somatomedin or growth hormone-insulin-like growth factor axis may be involved (13–15). Because malnutrition and related episodes of infection have different effects on the hormonal milieu, it is possible that hormonal disturbances are a secondary manifestation of malnutrition. There may also be novel metabolic effects in malnourished children with HIV infection.

TABLE 3
Mean Z-Scores According to HIV Disease Category

	E (n = 16)	N (n = 8)	A–C (n = 26)	H (n = 20)
Weight-for-age	−0.08	−0.82	−0.52	−9.60
Height-for-age	−0.41	−1.63	−1.21	−9.50
Weight-for-height	0.37	−0.10	−0.09	−2.60

Generally, a person who is receiving inadequate nutrition will use endogenous fat stores after short-term carbohydrate sources are depleted. This results in weight loss and a wasted appearance as subcutaneous fat stores are diminished and lean body mass (LBM) is relatively preserved. Adult patients with HIV infection have altered lipid metabolism (16–18). Furthermore, preliminary data suggest that accelerated protein breakdown and negative nitrogen balance occur in adult patients with AIDS when they have secondary infections (19). Stein et al. (20) have shown decreased rates of protein synthesis in stable adult patients with AIDS. The failure to adequately use endogenous fat stores combined with increased nitrogen losses and decreased protein synthesis results in a loss of LBM and a relative preservation of fat stores (21). In children, linear growth may be used as a clinical indicator of the accretion of LBM. Disruption of the LBM compartment in a child leads to a rapid cessation of linear growth as we observed in our patient population.

After the loss of LBM in AIDS patients, fat stores are ultimately used. It is at this advanced stage of AIDS that traditional indicators of wasting, such as a depressed weight-for-height, are seen. Waiting to institute aggressive nutritional support until this advanced stage of AIDS-induced malnutrition may lead to suboptimal results. Many of our patients in Category H died despite aggressive nutritional support. These deaths were caused by the underlying disease and were not complications related to nutritional therapies. Safety of such therapies has been established in adults and children (22–24). Similar to the children in our study, in a study by Henderson et al. (24), they were able to replete body weight in severely wasted children with HIV infection but were not able to show an effect on linear growth during the observation period. Earlier intervention or continued support may have reversed this LBM effect.

In conclusion, our data suggest that the earliest expression of malnutrition in children with HIV infection is a decline in linear growth although the weight-for-height is relatively well preserved for a prolonged time. This pattern can result in a false impression of adequate nutrition and requires that careful attention be given to longitudinal growth data of a child with HIV infection. Evidence of the failure of linear growth before clinical wasting is apparent may be an absolute indication for aggressive nutritional support. Waiting for the onset of frank malnutrition may yield poor results despite aggressive nutritional support. We believe that it is likely that nutritional therapy will be most successful when it is instituted to prevent, or at least to treat the first signs of malnutrition.

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Effect of Antiviral Drugs and Hematopoietic Growth Factors on *in Vitro* Erythropoiesis

RONA S. WEINBERG, PH.D.,[1] EILEEN D. CHUSID, PH.D.,[2] YELENA GALPERIN, M.D.,[1] JOANN C. THOMSON, M.D.,[2] TONY CHEUNG, M.D.,[2] AND HENRY S. SACKS, PH.D., M.D.[2]

Abstract

Background: The purpose of these studies was to improve our understanding of nucleoside analog, antiviral, drug-induced anemia in HIV infection.

Methods: Peripheral blood erythroid progenitor cells (BFU-E) from HIV-positive (HIV+) patients and normal donors were compared in methylcellulose cultures with erythropoietin, with and without antiviral drugs, and with and without the hematopoietic growth factors, stem cell factor (SCF), hemin, and interleukin-3 (IL-3).

Results: Normal numbers of BFU-E-derived colonies were observed in cultures from HIV+ patients (mean ± 1 SD BFU-E/10^5 cells plated: normal = 14.1 ± 7.9, HIV+ = 17.2 ± 14.2, $p = 0.39$). The antiviral drugs zidovudine (AZT), dideoxyinosine (ddI), and didehydrodideoxythymidine (d4T) all inhibited erythroid colonies in HIV+ and normal cultures. AZT was the most erythropoietically inhibitory drug (AZT ID_{50}, mean ± 1 SD for normal cultures = 2.64 ± 4.15 μM, for HIV+ cultures = 6.28 ± 10.79 μM, $p = 0.24$). Hematologic toxicity was less with ddI and d4T. However, doses of ddI and d4T $\leq$ 10 μM inhibited colony growth in 9/14 and 8/12 cultures, respectively, from HIV+ patients.

Conclusions: Stem cell factor (SCF), hemin, and interleukin-3 (IL-3) increased colony growth in HIV+ and normal cultures. In control cultures, hematopoietic growth factors added singly increased growth 1.3- to 8-fold. Hematopoietic growth factors increased growth even in cultures containing antiviral drugs. In some instances growth factors restored growth to control levels. SCF, hemin, and IL-3 were most effective when combined. **Key Words:** AZT, ddI, d4T, stem cell factor, hemin, erythropoiesis

ANEMIA IS A SIGNIFICANT complication of human immunodeficiency virus (HIV) infection which often becomes more severe with the progression of the disease (1–4) and sometimes curtails antiviral drug therapy with zidovudine (AZT) (5). The etiology of this anemia is not well understood but seems to be multifactorial. Suggested pathophysiology includes: HIV infection of hematopoietic stem/progenitor cells (S/PC) (6–9), inhibition of S/PC proliferation by HIV-derived proteins (7, 8, 10–12), indirect inhibition of S/PC by HIV-infected cytokine-producing accessory cells (9, 13), immune mediated suppression (14), and inhibition by antiviral drug therapy (5, 15–17).

Hematopoiesis in HIV infection has been studied extensively *in vitro.* Data are sometimes conflicting and thus controversial. Colonies derived from progenitor cells have been shown to be normal (18–21) or reduced in number (7, 10, 21–26). Disease status and reduced numbers of colonies were correlated in some studies (23, 24) but were uncorrelated in other studies (6, 25).

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[1]Polly Annenberg Levee Hematology Center and [2]the Thomas C. Chalmers Clinical Trials Unit, Department of Medicine, Mount Sinai School of Medicine, New York, NY 10029. Address correspondence to Rona S. Weinberg, Ph.D., Division of Hematology, Box 1079, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, New York, NY 10029.

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HIV infection of hematopoietic progenitor cells could result in either abnormal proliferation and differentiation or death of progenitor cells. CD34+ progenitor cells from HIV-positive (HIV+) patients were reported both as infected with HIV (6, 27) and not infected with HIV (HIV–) (10, 23, 28, 29). Similarly, colonies from HIV+ patients were either HIV+ (30) or HIV– (10, 28). Attempts to infect normal progenitor cells with HIV viral isolates suggest that progenitor cells can be infected with HIV, but HIV does not replicate within these cells (9, 18, 27). In other studies, HIV did not infect normal progenitor cells (11, 23, 29).

Hematologic abnormalities in HIV infection are also caused by treatment of the disease. The nucleoside analog azidothymidine (3′-azido-3′-deoxythymidine, zidovudine, or AZT) is the primary antiviral therapy for HIV infection (31). It inhibits viral replication by interfering with viral reverse transcriptase (32). Unfortunately, it also inhibits DNA synthesis in human cells, and treatment with AZT often results in severe dose-limiting, transfusion-dependent anemia and neutropenia (31). AZT also inhibits hematopoietic colonies *in vitro* (33–44). Dideoxyinosine (ddI), another nucleoside analog reverse transcriptase inhibitor, is also therapeutic in HIV infection (45, 46). *In vivo* ddI is not as toxic hematologically as AZT, but ddI does inhibit hematopoiesis *in vitro* (38, 41, 43). Other nucleoside analogs with antiviral activity such as dideoxycytidine (ddC), dideoxyadenosine (ddA), and didehydrodideoxythymidine (d4T) also inhibit hematopoiesis *in vitro* (34, 35, 38, 44, 46).

Hematologic status of HIV+ patients treated with AZT was improved with erythropoietin and/or granulocyte macrophage colony-stimulating factor (GM-CSF) therapy (47–54). In cultures of normal hematopoietic cells GM-CSF (55), lithium chloride (39), interleukin-3 (40), stem cell factor (SCF) (42), and hemin (37) all improved colony growth in cultures containing AZT. SCF also improved growth of colonies from HIV+ patients in AZT-containing cultures (42).

To improve our understanding of anemia in HIV infection we compared erythroid progenitors from HIV+ patients and healthy individuals *in vitro* regarding colony growth and inhibition by AZT, ddI, and d4T. In addition, the ability of hemin, SCF, and IL-3 (alone and in combination) to overcome inhibitory doses of these antiviral drugs was examined.

Materials and Methods

Peripheral blood (PB) was obtained from 21 healthy adults (18 male, 3 female) and 29 HIV+ adult patients (24 male, 5 female); all were between 20 and 45 years of age. All procedures were approved by the Institutional Review Board of the Mount Sinai School of Medicine. Three of the HIV+ patients were previously untreated. Other patients had received AZT, ddI, ddC, alone or in combination. All patients were participants in AIDS Clinical Trial Group (ACTG) protocols which are still blinded. In HIV+ patients, white blood cell counts ranged from 1.5 to 7.9, lymphocyte levels ranged from 6% to 57%, T4 cells from 5 to 360, T8 cells from 31 to 3512, and T4/T8 ratios from 0.01 to 0.45.

Blood samples were collected into heparinized vacutainer blood collection tubes (Becton-Dickinson, Rutherford, NJ). To prepare mononuclear cells (MNC) blood was diluted 1:1 with alpha medium without nucleosides (GIBCO Laboratories, Grand Island, NY) layered onto an equal volume of Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ) and centrifuged at 450*g* for 30 min at 18°C (56).

Erythroid Cultures. MNC were cultured in methylcellulose according to methods described previously (56). Each milliliter of culture contained 3×10^5 MNC, 0.8% methylcellulose (Fisher Scientific Co., Pittsburgh, PA) in alpha medium, 30% fetal bovine serum (Armour Pharmaceutic Intergen Company, Purchase, NY), 1% BSA (Cohn fraction IV; Sigma Chemical Co.), 10^{-4} M 2-mercaptoethanol (Sigma Chemical Co.), 0.1 U penicillin, 0.1 μg streptomycin/mL (GIBCO Laboratories). Cultures were done in triplicate in 0.3-mL volumes in NUNC four-well dishes (Intermed, Rosklide, Denmark) and incubated at 37°C in 4% CO_2. Colonies derived from burst-forming units erythroid (BFU-E) were counted on day 13 or 14 using a Bausch & Lomb stereozoom dissecting microscope (Bausch & Lomb, Rochester, NY). BFU-E/mL = BFU-E/100,000 cells plates plated × MNC/mL of blood.

Hematopoietic Growth Factors (HGFs). All cultures contained 2 U erythropoietin (Ep, kindly provided by Ortho Pharmaceutical Corp, Raritan, NJ)/mL. Interleukin-3 (IL-3) (Amgen Biologicals, Thousand Oaks, CA) was added to cultures at a dose of 50 U/mL. SCF (kindly provided by Dr. K. Zsebo, Amgen Biologicals) was added to cultures at a dose of 100 ng/mL. Hemin (Amresco, Salon, OH) was prepared as described previously (57) and added to cultures at a concentration of 100 μM.

Antiviral Drugs. AZT was kindly provided by Burroughs Wellcome Co. (Research Triangle Park, NC). ddI and d4T were much appreciated gifts of Bristol Myers Squibb Company (Wallin-

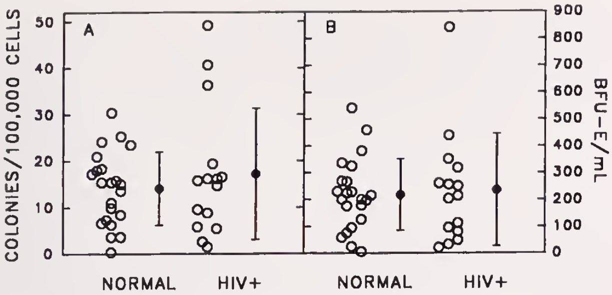

Fig. 1. Blood BFU-E-derived colonies. Growth potential of blood BFU-E-derived colonies from healthy and HIV+ adults. A, Colonies/100,000 cells plated; B, colonies/mL of blood. Each ○ is a different donor; ● is the mean; vertical lines indicate ± 1 SD.

ford, CT). All antiviral drugs were dissolved in Dulbecco's phosphate-buffered saline (GIBCO Laboratories), filter sterilized with 0.2 μM Millipore filter (Millipore, Bedford, MA) and diluted serially. Drugs were prepared fresh for each experiment and added to methyl cellulose cultures in doses ranging from 0 to 100 μM.

Statistical Analysis. Data from triplicate cultures were compared by Student's *t*-test.

Results

The number of MNC cells recovered from the blood of normal and HIV+ individuals was similar (mean ± 1 SD: HIV+ $1.42 \times 10^6 \pm 0.55 \times 10^6$ cells/mL; normal = $1.57 \times 10^6 \pm 0.61 \times 10^6$ cells/mL, $p = 0.44$).

BFU-E-derived colony growth from healthy and HIV+ donors on day 13 of culture is compared in Figure 1. The numbers of BFU-E/10^5 cells plated in normal and HIV+ cultures were similar. The means, ranges, and *p* values for these experiments are in Table 1. The number of MNC recovered from Ficoll-Hypaque centrifugation can vary with the donor's white blood cell count, particularly from HIV+ patients. Therefore, BFU-E/mL of blood provides a more physiologic comparison. The number of BFU-E/mL of blood was similar in normal and HIV+ cultures (Table 1).

AZT dose-response curves of BFU-E from healthy and HIV+ individuals are shown in Figure 2. Addition of 1 μM AZT inhibited growth 16 to 100% (mean, 67%) in 15 of 15 normal cultures. In 12 of 14 cultures from HIV+ patients, 1 μM AZT inhibited growth 40 to 84% (mean, 53%), the same dose stimulated growth in 1 of 14 and had no effect of 1 of 14 HIV+ cultures. The mean AZT ID_{50} (i.e., the dose of AZT which resulted in 50% inhibition of BFU-E) was 2.64 ± 4.15 μM in normal and 6.28 ± 10.79 μM HIV+ cultures ($p = 0.24$) (Table 1).

ddI dose-response curves of BFU-E from healthy and HIV+ individuals are illustrated in Figure 3. Doses of ddI ≤10 μM inhibited BFU-E in 10 of 14, stimulated growth in 3 of 14, and had no effect on the number of BFU-E in 2 of 14 normal cultures. In HIV+ cultures, ≤10 μM ddI inhibited BFU-E in 9 of 14, stimulated BFU-E in 4 of 14, and had no effect on BFU-E in 1 of 14 cultures (Table 1).

d4T dose-response curves of BFU-E from healthy and HIV+ individuals are shown in Figure 4. Doses of d4T ≤10 μM inhibited BFU-E in 11 of 11 normal cultures. In HIV+ cultures, ≤10 μM ddI inhibited BFU-E in 8 of 12, stimulated BFU-E in 2 of 12, and had no effect in BFU-E in 2 of 12 (Table 1).

The effects of AZT, ddI, and d4T were com-

TABLE 1

Characteristics of Blood BFU-E from Normal Donors and HIV+ Patients

	Normal	*n*	HIV+	*n*	*p*
Colonies/100,000 cells					
Mean	14.1 ± 7.9	22	17.2 ± 14.2	15	0.39
Range	0.4–30.4		1.4–49.1		
BFU-E/mL					
Mean	218.9 ± 134.1	22	235.4 ± 209.5	15	0.77
Range	5.1–541.7		21.0–843.0		
AZT, ID_{50} (μM)					
Mean	2.64 ± 4.15	15	6.28 ± 10.79	14	0.24
Range	0.0006–11.5		0.09–25.2		
ddI					
≤10 μM inhibited growth	10	14	9	14	
≤10 μM stimulated growth	2	14	4	14	
≤10 μM no effect	2	14	1	14	
d4T					
≤10 μM inhibited growth	11	11	8	12	
≤10 μM stimulated growth	0	11	2	12	
≤10 μM no effect	0	11	2	12	

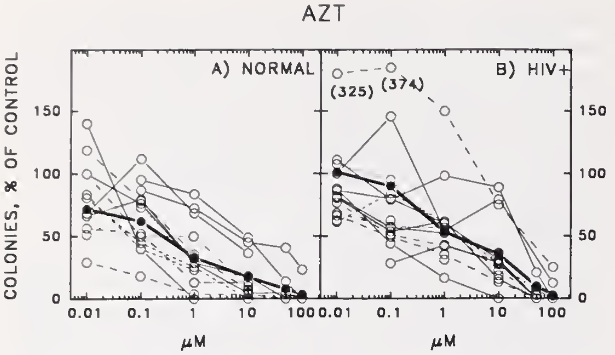

Fig. 2. AZT dose-response curves. Blood BFU-E-derived colonies from (A) normal and (B) HIV+ adults. Data were normalized as the percent of growth in controls (2 U Ep/mL). Each curve (○) represents a different donor; each point is the mean of triplicate cultures. ● is the mean of all curves.

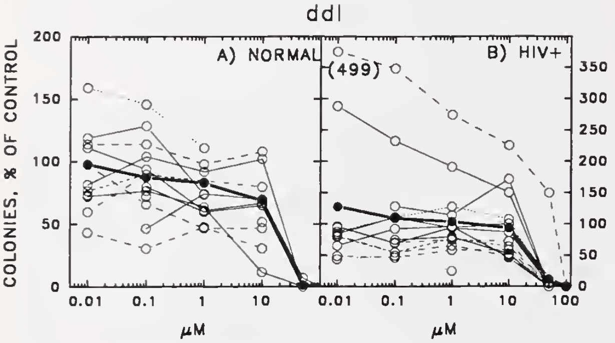

Fig. 3. ddI dose-response curves. Blood BFU-E-derived colonies from (A) normal and (B) HIV+ donors. Data were normalized as the percent of growth in controls (2 U Ep/mL). Each curve (○) represents a different donor; each point is the mean of triplicate cultures; ● is the mean of all curves.

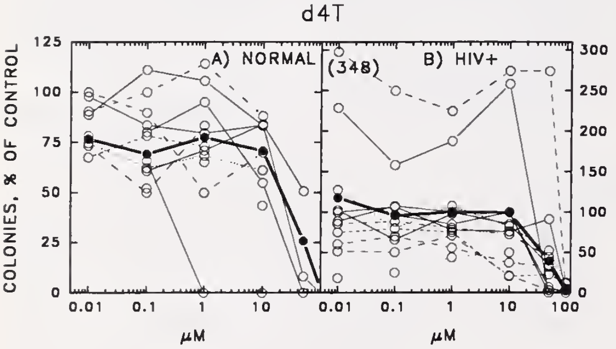

Fig. 4. d4T dose-response curves. Blood BFU-E-derived colonies from (A) normal and (B) HIV+ adults. Data were normalized as the percent of controls (2 U Ep/mL). Each curve (○) represents a different donor; each point is the mean of triplicate cultures; ● is the mean of all curves.

pared in 12 HIV+ cultures (Table 2). AZT (≤10 μM) always inhibited BFU-E. In each of 2 of 12 cultures obtained from the same patient, both ddI and d4T stimulated growth. The results of exposure to ddI and d4T in 4 of 12 cultures of cells from the same patient were discordant, either showing no effect or inhibition in three, and stimulation or inhibition in one.

TABLE 2

Effect of Antiviral Drugs[a] on BFU-E in HIV+ Cultures

Patient	AZT	ddI	d4T
1	I	S	S
2	I	S	S
3	I	I	I
4	I	S	I
5	I	I	I
6	I	I	I
7	I	I	I
8	I	NE	I
9	I	I	I
10	I	I	I
11	I	I	NE
12	I	I	NE

[a] Doses of drugs were ≤10 μM. I, inhibited; S, stimulated; NE, no effect.

The effects of SCF on BFU-E growth in cultures from HIV+ patients are shown in Figure 5. In cultures containing erythropoietin without antiviral drugs, SCF increased BFU-E 1.1- to 1.6-fold. AZT (Fig. 5, A and B), ddI (Fig. 5, C and D),

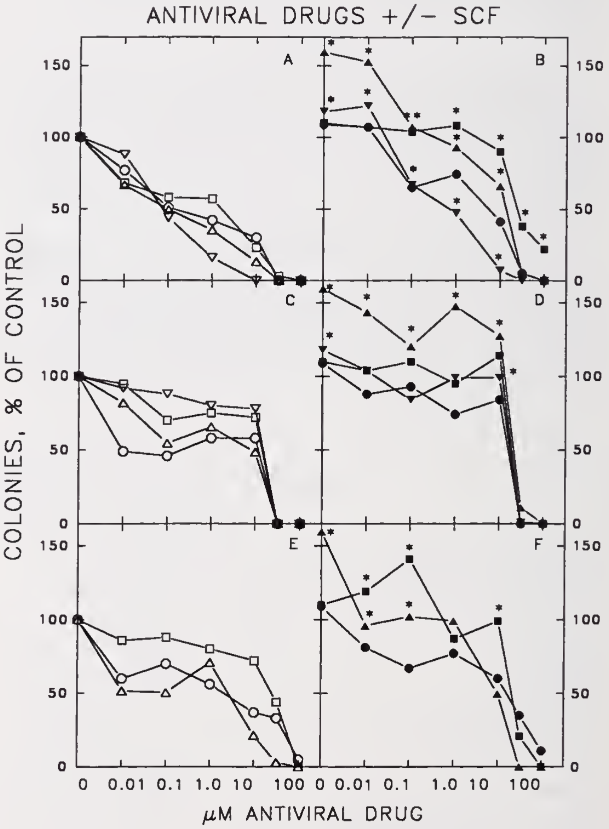

Fig. 5. Effect of SCF. Antiviral drug dose-response curves without (open symbols) and with (filled symbols) SCF. A and B, AZT; C and D, ddI; E and F, d4T. Each symbol represents a different HIV+ donor; curves with the same symbol are the same HIV+ donor. Data were normalized as the percent of controls (2 U Ep/mL). Each point is the mean of triplicate cultures. $^*p < 0.05$ compared with the same dose of antiviral drug without SCF.

and d4T (Fig. 5, E and F) all inhibited growth of BFU-E even in cultures containing SCF; however, cultures with SCF had more BFU-E than corresponding doses of antiviral drugs. SCF increased growth of BFU-E even at high doses of antiviral drugs (i.e., up to 10 μM antiviral drug). AZT was the most inhibitory antiviral drug and the effects of SCF were more dramatic in cultures with AZT than in those with ddI or d4T alone. Similar results were obtained in three normal cultures (data not shown).

The effects of IL-3 on BFU-E growth in cultures from HIV+ patients are shown in Figure 6. In cultures without antiviral drugs, IL-3 increased BFU-E 1.3- to 3.1-fold. As with SCF, IL-3 did not prevent inhibition of BFU-E by antiviral drugs; however, BFU-E numbers were higher in cultures with IL-3 than at corresponding doses of antiviral drugs without IL-3. IL-3 was more effective at lower doses of antiviral drugs (i.e., ≤0.1 μM). Observations were similar in three normal cultures (data not shown).

The effects of hemin on BFU-E growth in cultures from HIV+ patients are shown in Figure 7. In some control cultures without antiviral drugs, hemin increased BFU-E 1.5- to 8-fold. As with SCF and IL-3, hemin did not prevent inhibition of BFU-E by antiviral drugs; however, colony numbers were higher in cultures with hemin than in cultures with corresponding doses of antiviral drugs alone. Similar data were obtained from cultures of healthy donors (data not shown).

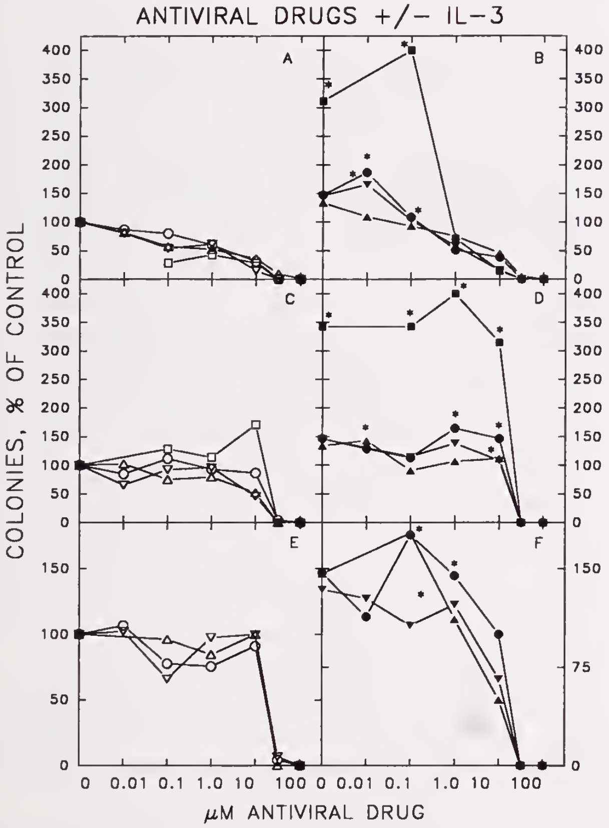

Fig. 6. Effect of IL-3. Antiviral drug dose-response curves without (open symbols) and with (filled symbols) IL-3. (A, B) AZT; (C, D) ddI; (E, F) d4T. Each symbol represents a different HIV+ donor; curves with the same symbol are the same HIV+ donor. Data were normalized as the percent of controls (2 U Ep/mL). Each point is the mean of triplicate cultures. *$p < 0.05$ compared with the same dose of antiviral drug without IL-3.

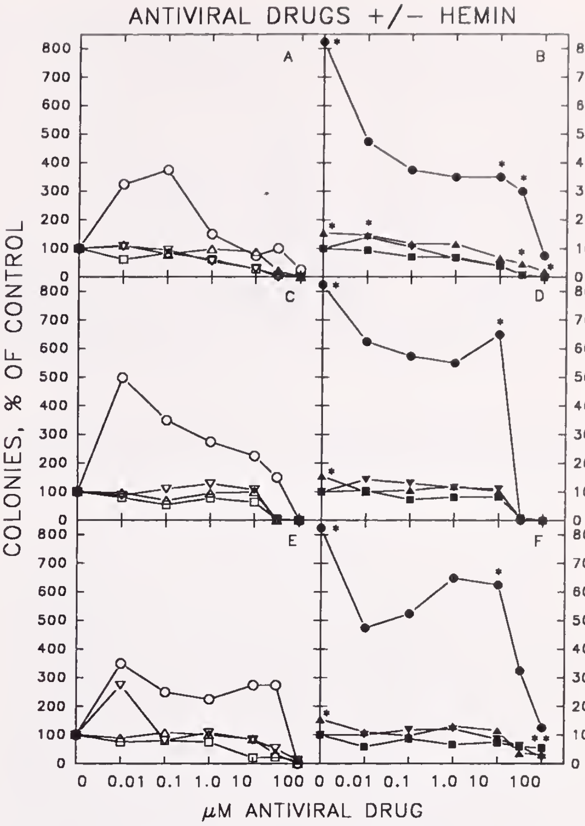

Fig. 7. Effect of hemin. Antiviral drug dose-response curves without (open symbols) and with (filled symbols) hemin. (A, B) AZT; (C, D) ddI; (E, F) d4T. Each symbol represents a different HIV+ donor; curves with the same symbol are the same HIV+ donor. Data were normalized as the percent of controls (2 U Ep/mL). Each point is the mean of triplicate cultures. *$p < 0.05$ compared with the same dose of antiviral drug without IL-3.

The combined effects of multiple hematopoietic growth factors were examined in three HIV+ cultures. Data from a representative experiment are shown in Figure 8. AZT, ddI, and d4T (either 1 μM or 10 μM) significantly inhibited *in vitro* erythropoiesis. Additions of hemin or SCF alone led to significant increases in BFU-E growth. Cultures with antiviral drugs and hemin or SCF often had more BFU-E than control cultures without antiviral drugs. At the doses of antiviral drugs chosen for this group of experiments IL-3 was not effective in improving BFU-E growth. Combining hemin, SCF, and IL-3 dramatically increased the number of BFU-E up to 6-fold compared with controls. The number of BFU-E with multiple growth factors was greater than the

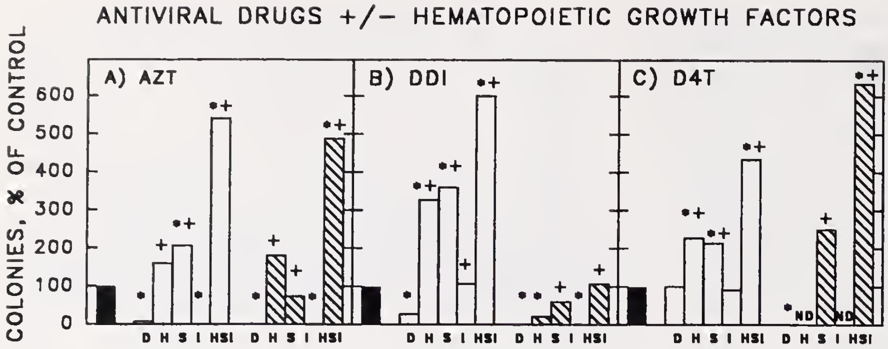

Fig. 8. Effect of multiple hematopoietic growth factors. Effect of hemin (H), SCF (S), and IL-3 (I) alone and in combination on cultures from HIV+ patients containing inhibitory doses of antiviral drugs (D). A, AZT; B, ddI; C, d4T; ■, control, 2 U Ep/mL; □, 1 μM antiviral drug; ▨10 μM antiviral drug. Data were normalized as the percent of controls (2 U Ep/mL). Each bar is the mean of triplicate cultures. +$p < 0.05$ compared with the same dose of antiviral drug without hematopoietic growth factors; *$p < 0.05$ compared with control (2 U Ep/mL).

combined number of BFU-E with each growth factor alone.

Discussion

In these studies the number of peripheral blood erythroid progenitor cells was similar in cultures from HIV+ and healthy individuals. Although normal numbers of bone marrow progenitor cells in HIV infection have been reported previously (18–21), others have observed fewer than normal progenitor cells (7, 10, 21–26). The reasons for these differences are not clear. Correlation between disease status and the number of hematopoietic progenitor cells was found in some studies (23, 24) but not in others (6, 25).

AZT inhibited growth in all cultures. The range and mean of AZT ID_{50} values were similar to those reported previously for normal (33, 35, 37, 40, 43, 44) and HIV+ (44) cultures. Normal cultures were more sensitive to AZT than HIV+ cultures suggesting inherent differences between erythropoiesis in HIV+ patients and healthy individuals.

ddI and d4T both inhibited erythropoiesis in some but not all normal and HIV+ cultures. Inhibition was usually less than that observed with AZT. In some patients either ddI or d4T (but not both) was inhibitory. In cultures from three HIV+ patients, d4T and/or ddI actually stimulated growth of erythroid colonies, perhaps by inhibiting production of inhibitory cytokines or proteins by HIV+ accessory cells. Thus, hematopoietic cultures may be useful to identify drugs, or combinations of drugs, which will produce more or less hematologic toxicity for individual HIV+ patients.

SCF is a potent stimulator of hematopoiesis (58–65). Unlike other hematopoietic growth factors such as GM-SCF and IL-3 which increase HIV replication *in vitro* (66–73), SCF does not influence HIV replication (42). SCF was effective in increasing erythropoiesis in cultures with and without inhibitory doses of antiviral drugs.

In cultures from healthy individuals, and patients with hemoglobinopathies and leukemia, hemin stimulates proliferation of erythroid, myeloid, and lymphoid cells (74–79). *In vivo* and *in vitro* heme abrogated AZT inhibition of erythropoiesis and myelopoiesis (37, 80, 81), and synergized with the antiviral activity of AZT (81). Our data confirm that hemin stimulates erythroid colonies in HIV+ cultures with and without inhibitory doses of AZT. We also demonstrated its effectiveness in augmenting erythropoiesis inhibited by ddI and d4T.

Previous reports have indicated that AZT inhibition of erythroid progenitor cells *in vitro* is caused at least in part by decreased heme metabolism and that this effect can be overcome by the addition of hemin (80). Whether ddI and d4T act similarly is unknown. However, hemin did improve BFU-E growth in cultures with inhibitory doses of ddI and d4T. Alternatively, antiviral drugs may interfere with the production of stimulatory hematopoietic growth factors or stimulate the production of inhibitory hematopoietic growth factors by accessory cells (such as T cells and monocytes). Our studies do not indicate whether antiviral drugs and hematopoietic growth factors act directly on progenitor cells or indirectly via accessory cells. Studies investigating these questions are underway in our laboratory using CD34+-enriched progenitor cells and enriched populations of T cells and monocytes.

IL-3 increased growth of normal bone marrow colonies in cultures containing AZT (40). However, in our studies, IL-3 did not abrogate antiviral drug inhibition of erythroid colonies as effectively as SCF or hemin.

The combination of hemin, SCF, and IL-3 *in vitro* provided the greatest stimulation of erythropoiesis in cultures from HIV+ patients. These data agree with reports regarding effects of hematopoietic growth factors in other syndromes of bone marrow failure (65, 82) and suggest that multiple hematopoietic growth factors should be used in the therapy of anemic HIV+ patients.

In summary, erythropoiesis in HIV+ and normal cultures was similar. AZT was the most inhibitory antiviral drug *in vitro;* however, ddI and d4T also inhibited the growth of erythroid colonies in HIV+ and normal cultures. Differences in *in vitro* response to AZT, ddI, and d4T suggest that *in vitro* hematopoietic cultures may be useful in predicting the least hematologically toxic therapy for each patient. SCF and hemin are potent stimulators of erythropoiesis even when inhibitory antiviral drugs are present, and are most effective when combined. Thus, anemic HIV+ patients treated with AZT or other nucleoside analog antiviral drugs may benefit from treatment with SCF in combination with other hematopoietic growth factors.

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Diagnosis and Management of Tuberculosis in Patients with HIV Infection

David N. Rose, M.D., Steven Vetter, and Alexandra Gurtman, M.D.

Human immunodeficiency virus (HIV) infection modifies the epidemiology and clinical manifestations of *Mycobacterium tuberculosis* infection and complicates the diagnosis and management of tuberculosis (TB). The diminished cell-mediated immunity caused by HIV infection is now considered to be the most powerful risk factor for the activation of latent *M. tuberculosis* infection (1). Furthermore, persons with HIV infection are especially susceptible to newly acquired *M. tuberculosis* disease. Patients who are immunodeficient from HIV infection manifest active TB atypically, with anergy, unusual chest radiographic patterns, and high rates of extrapulmonary disease. We review here these special features and their effect on the diagnosis and management of TB.

The Effect of HIV Infection On the Epidemiology of TB

Persons with HIV infection are more likely to develop active disease from newly acquired *M. tuberculosis* infection than persons not infected with HIV. Persons not infected with HIV have approximately a 30% risk of becoming infected with *M. tuberculosis* after a close household exposure (2) and if infected they face an approximate 3% risk of active TB in the first year after infection and lower risks thereafter (3, 4). The much higher risks conferred by HIV infection have been alarmingly demonstrated by analyses of institutional TB outbreaks (5–13). Di Perri and coworkers (6) documented the rapid progression of newly acquired TB in hospitalized patients with HIV infections. Among 18 patients with negative tuberculin skin tests exposed to an active, hospitalized TB case, eight developed active TB, seven within 60 days of exposure. The mean CD4 lymphocyte count was lower in those who developed TB than in those who did not (5). Daley and coworkers (12) described an investigation of a TB outbreak in a congregate-living facility for persons with HIV infection. Using restriction-fragment-length polymorphisms, they confirmed transmission of the strain to 11 (37%) of 30 exposed persons within 106 days. One patient developed the active disease 4 weeks after exposure. Another 4 (13%) of the 30 patients developed positive tuberculin skin tests (12).

Persons with HIV infection who have latent *M. tuberculosis* infection also have a higher risk of reactivation disease than persons not infected with HIV. Persons not infected with HIV with positive tuberculin skin tests for more than 7 years have a TB reactivation risk of approximately 0.07 to 0.1 cases per 100 patient-years (3, 4). Selwyn and co-workers (14) documented the far higher rate of HIV infection among intravenous drug users in a methadone-maintenance treatment program in the Bronx, New York. During a mean follow-up of 22 months, 7 (14%) of 49 HIV-infected, tuberculin-positive (⩾10 mm of reactivity) persons developed TB (7.9 cases per 100 patient-years), but none of the 62 HIV-uninfected, tuberculin-positive persons developed TB (14). Subsequent studies in the Bronx and in Madrid, Spain found that HIV-infected, tuberculin-positive (⩾5 mm of reactivity) patients developed TB at rates of 9.7 to 10.4 cases per 100 patient-

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Departments of Community Medicine and Medicine and the AIDS Center, Mount Sinai School of Medicine. Address correspondence to David N. Rose, M.D., Box 1009, Mount Sinai Medical Center, New York NY 10029.

years. HIV-infected anergic patients in these studies developed TB at rates of 6.6 to 12.4 cases per 100 patient-year (15, 16). These studies suggest that the TB reactivation risk in patients with HIV infection is approximately 100 times greater than in patients not infected with HIV. Some studies found that patients with HIV infection were more likely than patients not infected with HIV to have multidrug-resistant (MDR) *M. tuberculosis* organisms (17–19). Other studies did not confirm the association (20, 21).

The Effect of HIV Infection On the Clinical Manifestations of TB

M. tuberculosis is relatively more virulent than most opportunistic pathogens of AIDS. *Pneumocystis carinii* pneumonia characteristically does not occur in persons with HIV infection until the CD4 count falls below 200 cells/μL, and *Mycobacterium avium* complex disease usually does not occur until the CD4 count falls below 100 cells/μL. TB, however, is found in patients with HIV infection who have a wide range of immune function, from very low to normal CD4 counts, with a median in the 300 to 400 cells/μL range (22). The greater virulence of *M. tuberculosis* in patients with HIV infection often results in active TB preceding other opportunistic infections. Approximately half of the cases of TB precede, one tenth are concurrent with, and the remainder follow other AIDS-defining conditions.

TB in patients with HIV infection who have no or mild immunodeficiency often presents with "typical" clinical manifestations. In moderate-to-severe immunodeficiency, three "atypical" features of TB are usually found: cutaneous anergy, "atypical" pulmonary manifestations, and high rates of extrapulmonary disease. These distinctions have not been fully documented, because most case series with immunological data are small.

Anergy, the absence of cutaneous delayed-type hypersensitivity (DTH), characterizes an advanced stage of HIV disease (23) and predicts the occurrence of AIDS-defining conditions (24). Cutaneous anergy is found not only in patients with latent *M. tuberculosis* infection but also in patients with active disease. In a San Francisco study of patients with TB, 61% of patients with AIDS had nonsignificant tuberculin skin test responses (<10 mm induration), whereas only 9% of patients without AIDS had nonsignificant tuberculin skin test responses (25). Similar findings were found among patients with TB in another San Francisco study (20% patients with HIV infection versus 7% of patients without infection were anergic) (22), in a study in Patterson, New Jersey (80% vs 17%) (26), and in a study in Zaire (33% vs 7%) (27). Impaired DTH also diminishes the usefulness of the tuberculin skin test. For this reason, in 1990 the American Thoracic Society and the Centers for Disease Control and Prevention (CDC) recommended that the definition of a positive tuberculin skin test [using 5 Tuberculin Units (TU) of intradermal PPD] in persons with HIV infection be changed from the previous value of ≥10 mm of induration to a value of ≥5 mm of induration (28). Nevertheless, many persons co-infected with HIV and *M. tuberculosis* are anergic to tuberculin (29) and are at high risk for active TB (15, 16).

Pulmonary manifestations, reflected in chest radiograph patterns, range from upper-lobe disease with cavities in patients with no or mild immunodeficiency to lower-lobe disease, miliary patterns, an absence of cavities, intrathoracic adenopathy, and occasionally normal chest radiographs, in patients with moderate-to-severe immunodeficiency (6, 22, 25, 27, 30–36). In severely immunocompromised patients, cavitation is uncommon and adenopathy is found frequently (30, 34, 36–38). Adenopathy is an uncommon finding in immunocompetent adults with TB (36). TB in patients with HIV infection is not more infectious than among patients without HIV infection (39).

High rates of extrapulmonary TB (45–79%) are characteristic of patients with HIV infection (30, 40–42). In a report of TB among patients with AIDS in Newark, New Jersey between 1981 and 1985, 21 (79%) of 29 patients had extrapulmonary disease, in contrast to the extrapulmonary rate of 27% among all patients with TB in Newark and 16% among all patients with TB in the United States in 1984 (31). Extrapulmonary disease has been found in 60% of patients with AIDS/TB in San Francisco (25, 43) and 50% in New York City (37). In an HIV-seroprevalence study of 3,077 patients in 20 TB clinics nationwide, HIV infections were more frequent in patients with extrapulmonary TB (19.8%) and both extrapulmonary and pulmonary disease (15.6%) than in patients with pulmonary disease alone (10.2%) (44). Overall, HIV-infected patients were twice as likely to have extrapulmonary disease than were patients not infected with HIV (44). Similarly, in Zaire more patients with extrapulmonary disease were HIV-seropositive (45%) than patients with pulmonary TB (33%) (27). Furthermore, among patients with HIV infection, patients with extrapulmonary disease are likely to have lower CD4 counts than persons without extrapulmonary disease (22). In a study of patients with TB and HIV infection, extrapulmonary TB was found in 70% of patients with ≤100 CD4 cells/μL, 50% of patients with 101 to 200 CD4 cells/μL, 44% of patients with 201 to 300 CD4 cells/μL, and 28% of patients with >300 CD4 cells/μL (45).

Extrapulmonary disease in patients with HIV infection generally occurs in the lymphatic system, gastrointestinal and genitourinary tracts, or the bone marrow (22, 25, 30, 33, 37). Disseminated disease is also common, occurring in approximately one half of reported patients (22, 30). Central nervous system TB has appeared in approximately 3% to 10% of patients in an AIDS/TB series (14, 25, 31, 46). *M. tuberculosis* bacteremia, uncommon in patients not infected with HIV, is not unusual (25, 33, 47). Tuberculous pericarditis (31, 40, 48), peritonitis (14), rectal abscess (31), pancreatic abscess (49), and scrotal TB have also been reported (31).

Diagnosis of TB in Persons With HIV Infection

Diagnosis of TB in patients with HIV infection requires a higher index of clinical suspicion because of the high likelihood of tuberculin skin test anergy, "atypical" pulmonary manifestations, and extrapulmonary disease. Chest radiographs may be nonspecific and not suggestive of TB (50). Acid-fast smear and culture of available clinical specimens, including respiratory secretions, bronchial washings, lung tissue, pleural fluid, lymph node tissue, bone marrow, blood, urine, stool, brain biopsy, and cerebrospinal fluid, are essential elements of the investigation. Sputum smears in patients with HIV infection have similar (22, 43) or slightly lower sensitivity (40, 51) than smears in patients with TB who do not have HIV infection, approximately 50% to 60% (22, 34, 43), although higher in some series (33). Bronchoscopic biopsy and/or bronchoalveolar lavage fluid smear, culture, and pathology add to the sensitivity of sputum analysis (52). The polymerase chain reaction (PCR) has been used to diagnose TB from cerebrospinal fluid, endoscopic biopsy specimens, sputum, and bronchoalveolar lavage specimens. PCR has similar or greater sensitivity than cultures from the same specimens in several studies, with the advantage of having results within 48 hours (53). Despite the increased risk of cutaneous anergy in HIV infection, tuberculin skin testing is often helpful in diagnosis. Approximately 70% of patients with HIV infection who do not have an AIDS-defining condition and approximately 30% of patients with AIDS will respond to 5 TU PPD with ≥10 mm of induration (54). In a study of 479 patients with HIV, anergy was 4 and 15 times as likely for persons with CD4 lymphocyte counts of 200 to 400 cells/μL and <200 cells/μL, respectively, as for persons with ≥500 cells/μL (55). Two-stage tuberculin skin testing, however, only allowed diagnosis of an additional 19 (2.7%) of 709 patients with HIV infection who were tested for TB the second time (56).

Delayed diagnosis poses a hazard of nosocomial spread. Flora and co-workers (57) described a series of 11 patients for whom the diagnosis of TB was not suspected. Even in patients with "typical" manifestations of TB, many of the appropriate diagnostic tests were not performed, *P. carinii* pneumonia was the presumed diagnosis in nine of these patients but was confirmed in only one. The index case in the hospital outbreak described by Di Perri and co-workers (6) had a cough but a normal chest radiograph; antituberculous therapy was not begun until the 28th day of hospitalization because of the low index of suspicion of TB. In the MDR-TB outbreak in four hospitals in New York and Miami, the diagnosis of TB was delayed because chest radiographs did not show "typical" TB patterns (10).

Treatment and Prevention of TB in Persons with HIV Infection

The principles of initial TB treatment are the same for patients with HIV infection as for patients without HIV infection: promptly initiate effective multidrug antituberculosis therapy based on clinical and drug-susceptibility surveillance data and monitor clinical and sputum smear and culture status. All hospitalized patients with potentially infectious TB should be placed promptly in respiratory isolation rooms with negative air pressure relative to adjacent areas. Standard therapeutic regimens now include directly observed therapy (DOT).

Therapeutic Drug Regimens

Because of the increasing incidence of multidrug-resistant TB in the United States, the CDC recently issued updated recommendations for the treatment of TB. These recommendations include the need for *in vitro* drug susceptibility testing of *M. tuberculosis* isolates from all patients and reporting results to the health department, initial four-drug regimens for the treatment of TB, and directly observed therapy for persons with TB (58) (Table 1). The recommendations state that

TABLE 1

Regimen Options for the Initial Treatment of TB Among Children and Adults

TB without HIV Infection			
Option 1	Option 2	Option 3	TB with HIV Infection
Daily INH, RIF, and PZA for 8 wk followed by 16 wk of INH and RIF daily or 2–3 times/wk.[a] In areas where the INH resistance rate is not documented to be <4%, EMB or SM should be added to the initial regimen until susceptibility to INH and RIF is demonstrated. Treat for a minimum of 6 mo and/or 3 mo beyond culture conversion, whichever is longer.[b]	Daily INH, RIF, PZA, and SM or EMB for 2 wk followed by 2 times/wk[a] of the same drugs for 6 wk, and subsequently, with 2 times/wk[a] of INH and RIF for 16 wk.[b]	Three times/wk[a] INH, RIF, PZA, and EMB or SM for 6 mo.[b,c]	Options 1, 2, or 3 can be used, but treatment regimens should continue for a total of 9 mo and at least 6 mo beyond culture conversion.[b]

Abbreviations: DOT, directly observed therapy; EMB, ethqambutol; PZA, pyrazinamide; RIF, rifampin; SM, streptomycin; TB, tuberculosis.

[a] All regimens administered 2 or 3 times/week should be monitored by DOT.

[b] Consult a TB medical expert if the patient is symptomatic or has a positive smear or culture after 3 months.

[c] The strongest evidence from clinical trials is the effectiveness of all four drugs administered for the entire 6 months. There is weaker evidence that SM can be discontinued after 4 months if the isolate is susceptible to all drugs. The evidence for stopping PZA before the end of 6 months is equivocal for the 3 times/week regimen, and there is no evidence on the effectiveness of this regimen with EMB for less than the entire 6 months.

Source: CDC. Initial therapy for tuberculosis in the era of multidrug resistance: recommendations of the Advisory Council for the Elimination of Tuberculosis. MMWR 1993; 42(No. RR-7).

patients with HIV infection should be treated for a total of 9 months and for at least 6 months after sputum conversion, all regimens administered two or three times weekly should be monitored by DOT, and consultation with a TB expert is advised if the patient remains symptomatic or has a positive smear or culture after 3 months of therapy.

In several case series, antituberculosis therapy appeared to be as effective for patients with HIV infection as for patients without HIV infection (25, 38, 43), but patients with HIV infection may have higher rates of relapse (59). In a standard 6-month regimen of daily isoniazid (INH) and rifampin with the addition of pyrazinamide for the first 2 months, the mortality rate among patients with HIV infection was 6% but only 0.4% among patients without HIV infections. Mortality was 10% among patients with HIV infection who had CD4 counts <200 cells/μL. Among persons who completed the therapeutic regimens, however, the cure rates were 93% and 92% for patients with and patients without HIV, respectively (60).

Several retrospective and prospective series have shown a relapse rate from 0 to 15% after completion of therapy (61, 62). Perriens et al. (62) reported that failures and relapses were 2.6 times higher in patients with HIV infection because of both immunodeficiency and poorer compliance in those with HIV infection. Patients with pulmonary TB had a median time to sputum culture negativity of 10 weeks (43).

A regimen of INH, rifampin, pyrazinamide, and ethambutol daily for 4 months, followed by rifampin and INH twice weekly for 2 months was given to persons with HIV infection in a study comparing a 6-month regimen with a 12-month regimen. After the initial 6 months, patients were randomly assigned to receive either rifampin and INH or placebo for an additional 6 months. The relapse rate at 24 months was 1.9% among those patients who received long-course therapy, whereas the relapse rate was 9% among those receiving placebo for the second 6 months. Survival was not significantly different between those who received therapy for the additional 6 months and those who received placebo (62).

Treatment failure occurred in only 6% of patients with HIV infection treated with the 9-month regimen recommended by the CDC for drug-susceptible TB. No relapses occurred during 916 patient-months of observation, but 54% of patients died of nontuberculosis complications of HIV disease after a mean follow-up of 18 months after the diagnosis of TB (63).

High rates of drug toxicity have been reported among patients with HIV infection. Adverse drug reactions are usually associated with INH and rifampin, although occasionally they can be related to ethambutol and pyrazinamide administration. Different series report that up to 27% of patients with HIV infection have toxicity to one or more drugs and up to 18% *of those* require discontinuation (43, 62). In addition, because of polypharmacy in patients with AIDS, drug interaction is a concern. Anemia may be more prominent in patients who take antimycobacterial therapy and zidovudine (64). Serum levels of rifampin can be reduced by the coadministration of methadone.

Noncompliance with posthospitalization self-administered oral medicine is very common. In one study, only 11% of patients completed therapy (65). DOT has proven effective, even in populations at high risk for noncompliance. In one series the completion rate was 88% (66). DOT in patients with HIV infection resulted in 96% of patients completing 6 months of therapy versus 76% of those self-administering therapy. Survival at completion of therapy among those receiving DOT was 85% versus 57% among those self-administering antituberculosis therapy (67).

Prevention of TB Among Persons with HIV Infection

Persons with HIV infection should be screened routinely for TB infection with the tuberculin skin test. The CDC recommends that ≥5 mm induration 48 to 72 hours after 5 TU intradermal PPD be the criterion for a positive test in persons with HIV infection and persons with risk factors for HIV infection, whose HIV infection status is unknown but who are suspected of being infected (68). Patients with a positive test and pulmonary disease should be examined promptly and tested for active TB. Those without evidence of active TB most likely have latent infection and are at high risk for active infection in the near future. If there are no contraindications, such persons should be considered for INH preventive therapy (300 mg daily for 1 year) (28).

Several studies support INH preventive therapy in HIV-infected tuberculin reactors. In two studies of HIV-infected tuberculin reactors, 7.9 and 9.7 TB cases/100 person-years occurred among those who did not take preventive therapy, but no cases occurred among those who took preventive therapy (14, 15). These observations were confirmed recently in a clinical trial conducted among persons with HIV infection in Haiti. Tuberculin-positive persons who received a 12-month course of INH plus vitamin B6 developed active TB during 3 years at a rate of 1.7 cases/100 person-years. Persons who received vitamin B6 alone developed TB at a rate of 10.0 cases/100 person-years. Remarkably, INH preventive therapy was also delayed progression to HIV disease, AIDS, and death (69). Another clinical trial found that 15% of tuberculin-reactive, HIV-infected patients developed active TB during 3 to 27 months of follow-up (median, 9 months), despite completing 9 months of isoniazid preventive therapy, which suggests that the benefit of prophylaxis with INH is of short duration in patients with HIV infection. In addition 19% of patients had to discontinue therapy because of drug intolerance (70).

The CDC recently revised the recommendations on anergy testing and preventive therapy for HIV-infected persons (71). The revisions were based on three factors that were found to limit the usefulness of anergy testing: problems with standardization and reproducibility of the test, the low risk of TB associated with a diagnosis of anergy (72), and the lack of apparent benefit of preventive therapy for anergic HIV-infected persons. Two clinical trials confirm the last point (72, 73). A study in Kampala, Uganda demonstrated a high TB case rate in placebo recipients but an insignificant (17%) reduction in isoniazid recipients (73). A study in the United States found an unexpectedly low case rate (0.9 per 100 person years) in placebo recipients and a statistically insignificant 56% reduction (to 0.4 per 100 person years) in isoniazid recipients (72). The investigators concluded that preventive therapy would have minimal impact in reducing the number of TB cases.

The CDC has also recently issued preventive therapy guidelines (74) for HIV-infected patients and persons without HIV infection who have been exposed to an active case of MDR-TB. Included in the guidelines are recommended methods to estimate the likelihood of new infection with *M. tuberculosis* and with MDR-TB and considerations for preventive therapy decision-making.

Occupational settings raise several important issues of TB prevention (75). Tuberculin skin testing at 6-month intervals is advised for all workers with high levels of potential exposure to TB. Workers with risk factors for having HIV infection should be offered voluntary HIV testing after counseling (76). Workers with positive tuberculin skin tests and with immunosuppressing medical conditions, including HIV infection, are at higher risk than persons without HIV infection for reactivation TB (14–16). Such persons should be counseled about taking INH preventive therapy (75). Workers with immunosuppressing medical conditions, including HIV infection, are also at higher risk for newly acquired TB disease.

For this reason, infection control policies and procedures and engineering controls are even more important for immunosuppressed workers than for healthy workers (75). An outbreak at a Florida hospital was halted by implementing the CDC recommendations (77). The best protection for severely immunodeficient workers is to avoid exposure to patients with active TB. Therefore, severely immunodeficient workers should have the option available of voluntary transfer to areas with the lowest risk.

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Passive Immunization for the Treatment of HIV Infection

Jeffrey M. Jacobson, M.D.

Abstract

Neutralizing antibodies are a component of the immune response to acute human immunodeficiency virus infection. Levels of these antibodies diminish with the clinical progression of HIV disease. The results of several clinical studies of humoral passive immunization for advanced HIV disease suggest no clear antiviral or immune-enhancing effect. However, some studies reported delay in the occurrence of opportunistic infections and prolongation of life. Further studies are needed to more specifically define the role of humoral immune response in preventing and controlling HIV infection, and the potential for augmentation of these responses in prophylaxis and treatment. **Key Words:** acquired immunodeficiency syndrome, HIV, HIV antibodies, immunization, passive

The production of neutralizing antibodies is a part of the measurable, vigorous, immune response to acute human immunodeficiency virus (HIV) infection (1). This response contains, but usually does not eradicate, the infection. The levels of neutralizing antibodies decline (2–4), along with other immune functions (5–7), over time in those patients whose HIV disease progresses. How much neutralizing antibodies contribute to the immune protection of the host against the advance of the HIV infection is unclear at the moment. Equally unclear is what protection these antibodies might provide against initial infection.

Experiments in both the chimpanzee and SCID mouse models have demonstrated that animals can be protected from an HIV challenge with anti-HIV hyperimmune globulin and neutralizing monoclonal antibodies (8–12). These products have also neutralized *in vitro* several laboratory strains and clinical isolates of HIV (13–15).

Because all these observations suggest an antiviral effect, the potential for passive immunization with neutralizing antibodies to serve as therapy for advanced HIV disease has raised some interest. Theoretically, there are several mechanisms by which humoral immunity could either prevent or moderate HIV disease. As mentioned above, neutralizing antibodies could prevent infection of new cells by a virus (13, 14). Antibodies could also work through antibody-dependent cellular cytotoxicity (ADCC) (5, 16) or complement-mediated lysis (17, 18) of HIV-infected cells. Antibodies could saturate viral gp120 expressed on infected cell surfaces, and thereby inhibit fusion of cells and syncytia formation. This has been demonstrated with some monoclonal antibodies (19). In addition, antibodies could promote clearance of immunosuppressive viral proteins such as gp120. Soluble gp120 itself can down-regulate CD4 cell function and promote antibody- and cytotoxic T lymphocyte-mediated lysis of uninfected CD4 lymphocytes (20–24).

On the other hand, antibodies directed against gp120, particularly if they don't interfere

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From the Division of Infectious Diseases, Department of Medicine, Mount Sinai School of Medicine, One East 100th Street, New York, NY. Address correspondence to Jeffrey M. Jacobson, M.D., Director, AIDS Center Box #1009, Mount Sinai School of Medicine, One East 100th Street, New York, NY 10029.

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TABLE 1

Evidence for the Role of Neutralizing Antibodies in HIV Infection

1. Part of immune responses to acute infection.
2. Associated with nonprogression of disease.
3. *In vitro* neutralization of laboratory strains and clinical isolates.
4. Animal protection studies.
5. Human clinical trials.

TABLE 2

Theoretical Role of Antibodies in HIV Infection

1. Neutralization.
2. ADCC.
3. Complement-mediated lysis.
4. Inhibition of cell-cell fusion.
5. Promote clearance of immunosuppressive viral proteins.

Caveat: Enhancement of cell infection.

with CD4 binding, have enhanced some of these gp120-mediated immunosuppressive effects (20). Concerns have been expressed as well about the ability of some anti-HIV antibodies to enhance infection of cells by virus (25, 26) and thereby promote viral dissemination. This occurs through the F_c receptor- and complement receptor-mediated uptake of the antibody-virus complex into macrophages.

In a report published in 1988, Jackson et al. (27) infused plasma containing high anti-p24 antibody titers into six patients with advanced AIDS-related complex (ARC) and AIDS. In the period immediately after each infusion, there was a loss of HIV p24 antigenemia, a reduction in the fraction of subjects with positive plasma and lymphocyte HIV cultures, fewer opportunistic infections, and improvements in symptom score, weight, and Karnofsky performance score.

That same year, in Great Britain, Karpas et al. (28) reported similar results in an uncontrolled study of ten patients with AIDS and ARC who received monthly infusions of hyperimmune plasma. Viremia, as measured by p24 antigenemia and polymerase chain reaction (PCR) assay for HIV nucleic acid, was reduced dramatically. However, the PCR method for detecting cell-free HIV DNA used in Karpas' study was invalidated by a subsequent study done by the Transfusion Safety Study Group (29) involving the laboratory used by Karpas and his colleagues.

To further evaluate passive immunotherapy for HIV disease, in 1988 to 1990 we conducted a double-blinded, randomized controlled trial of human hyperimmune plasma (30) prepared using the methods described by Karpas et al. (31). Patients with stage IV HIV disease as defined by the Centers for Disease Control and Prevention (CDC) were studied.

Plasma donors had HIV infection, but were p24 antigen negative, had CD4 counts greater than 400, no clinical history of AIDS or ARC, and antibody titers against an HIV-infected cell line of ≥ 640 as determined by the immunoperoxidase method developed by Karpas. When Karpas conducted this rapid test, the results correlated well with neutralization activity, although we subsequently studied this in a blinded fashion and found poor correlation (32). Nevertheless, the plasma we infused had neutralization titers ranging from 1:158 to 1:446.

Four different B-propriolactone-treated plasma pools were created during the course of the study using from 6 to 11 different donors per pool. Those randomly assigned to the treatment arm of the study received 250 mL of HIV immune plasma intravenously every 4 weeks; members of the control group received 250 mL of HIV-antibody-negative plasma every 4 weeks.

Of 63 consecutive eligible subjects enrolled in the study, 31 were randomly assigned to the treatment group and 32 to control. Our patients had advanced disease, with a geometric mean CD4 lymphocyte count at baseline of 36 cells/mm^3 in the treatment population and 45 cells/mm^3 in the control arm of the study. We noted this to explain our negative results.

In both study groups, the geometric mean CD4 lymphocyte count did not increase after the start of treatment. Instead, it declined steadily in both populations with no significant difference in rate between them. In addition, there were no statistically significant differences between study groups in the effects on plasma or cell viral culture titers.

On the other hand, there was a statistically nonsignificant trend toward delayed occurrence of a new opportunistic infection in the treatment arm of the study, with the median time to the development of a new opportunistic infection after baseline of 52 weeks in the treatment group compared with 36 weeks in the control group. There also was a statistically nonsignificant trend toward improved survival. The median survival of the treatment group was 88 weeks, compared with 59 weeks in the control group. There were no significant differences between the groups in changes in trends of weight, Karnofsky performance score, or serum β_2-microglobulin levels.

In summary, although showing safety and raising the possibilities of improved survival and delayed occurrence of opportunistic infection, this passive immune product showed no appreciable antiviral or CD4 cell-enhancing activity in the population studied.

In 1992, a French team headed by Vittecoq conducted a nonblinded, randomized controlled trial (33) in which they infused heat-inactivated plasma from single donors with high p24 antibody titers into half of 18 subjects with CD4 counts less than 100 cells/mm^3. HIV-p24 antigen became undetectable by standard assay in all patients in the treatment group. No effects were seen on CD4 cell counts, weight, or Karnofsky performance score, but fewer opportunistic infections occurred in the treated subjects during the 6-month study. After the study infusions ended, the investigators noted fever, weight loss, and lower Karnofsky scores, which responded to retreatment in some of the patients receiving the immune plasma. However, these clinical parameters were not affected during the controlled study period. Nevertheless, the investigators raised the question of rebound worsening of disease when therapy is discontinued. We did not see this in our study, nor was it seen in a subsequent larger trial by Vittecoq et al. (34).

In 1994, Levy et al. (35) reported the results of a study in which 220 subjects with symptomatic HIV disease were double-blindly randomly selected for monthly infusions for 1 year of either 250 or 500 mL of HIV hyperimmune plasma, which had been pooled from HIV-infected donors with high anti-p24 antibody levels, or albumin as placebo. No clear benefit was detected in the study population as a whole. However, in subset analysis, a statistically significant improvement in CD4 lymphocyte counts relative to placebo was seen only in the subset of patients with 50 to 200 CD4 cells/mm^3 who received the high dose of plasma. The same subset was the only group to manifest trends toward improved survival and a reduction in p24 antigen levels. No other HIV load measurements were performed. No effects on opportunistic infections or β_2-microglobulin were seen.

French investigators followed up with a randomized, this time double-blind controlled trial in 82 subjects with AIDS in which patients received 300 mL of single-donor hyperimmune plasma or HIV-negative plasma every 2 weeks for 1 year (34). To be eligible, patients needed to be CDC stage IV and have $\leq$200 CD4 cells/mm^3. A significant effect on delaying the occurrence of a new opportunistic infection was found, along with a trend toward improved survival. No differences were detected between the two groups in CD4 cell counts or levels of interleukin-1, interleukin-6, or tumor necrosis factor.

Viral load studies gave mixed results (36). HIV-p24 antigen (both standard and immune complex dissociated) declined significantly in the treated group versus control. On the other hand, quantitative HIV-RNA as measured by PCR assay behaved the same in both groups of patients. It was reported that plasma, but not cellular, quantitative HIV cultures declined significantly in the treated group versus control group.

In an alternate approach, a Danish group immunized pigs with an HIV strain lysate and created a porcine hyperimmune globulin. This product, infused daily for 5 days into 14 subjects with HIV infection in an uncontrolled phase I study reported in 1992, resulted in improvements in symptoms, clearance of p24 antigenemia in those initially positive, and a delayed, although not early, improvement in CD4 cell counts (i.e., 4–6 months later) (37).

Two studies of monoclonal anti-HIV antibodies have appeared in the literature to date. A Swedish group headed by Jorma Hinkula recently reported a trial of two different murine monoclonal antibodies, both with neutralizing activity directed at the V3 loop of the IIIB strain, one of which also mediated ADCC (38). Eleven patients with AIDS were infused with one of the antibodies bimonthly for a 3-month period. The primary viral isolates of 9 of the 11 subjects were neutralizable by the antibodies *in vitro*. Five subjects demonstrated decreases in p24 antigenemia, and four had decreases in viremia as assayed by PCR. Although there were no changes in CD4 lymphocyte counts, T-cell lymphoproliferative responses to both mitogens and antigens were enhanced in most patients.

In a Swiss study, a chimeric mouse-human monoclonal antibody to the V3 loop of HIV-gp120 was given to 12 patients with advanced HIV disease (39). Various doses were infused at 3-week intervals for 24 weeks. Six patients experienced a significant decrease in viral load measures (two by branched chain DNA [bDNA] measurement of HIV-RNA, five by heat-denatured p24 antigen [HDp24Ag]) and three had an increase (one by bDNA, two by HDp24Ag). Only one patient had a significant reduction in both measures.

In conclusion, preliminary human trials suggest that there may be a clinical benefit (delayed opportunistic infections, improved survival) from the administration of anti-HIV hyperimmune plasma, but this needs to be established more firmly in larger well-controlled trials. There does not appear to be an effect on CD4 lymphocyte counts (although one study reported a positive effect in patients with counts of 50–200 cells/mm^3 and not in those with CD4 counts less than 50/

mm^3). A specific antiviral effect remains to be more clearly proven in controlled studies.

Further studies are needed to better define the role of passive humoral immunization in modifying the course of HIV infection, and regarding plasma studies, to determine whether there are other factors in plasma at work: cytokines, etc. We need to learn more about what role, if any, the various antibody-mediated immune responses have in the control of, and protection against, HIV infection, and what viral epitopes are important in these responses. Pathogenesis-oriented clinical studies should complement laboratory research in this regard. Hopefully, this information will help guide the effort toward developing both postexposure and preventive vaccine strategies.

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HIV-Associated Nephropathy

JONATHAN WINSTON, M.D., AND PAUL E. KLOTMAN, M.D.

Abstract

Background: Patients with HIV-1 infection are at risk for developing renal diseases with diverse etiologies. Acute renal failure occurs in up to 20% of hospitalized patients with HIV infection, and chronic renal disease of diverse etiology has been reported. The single most common cause of chronic renal insufficiency in HIV-1+ patients is HIV-associated nephropathy (HIVAN). Typical morphologic features include enlarged kidneys, microcystic tubule dilatation, tubulointerstitial inflammation, and focal and segmental glomerulosclerosis.

Methods: The pathogenesis, epidemiology, and treatment options for HIVAN are discussed. In studying the epidemiology of the disease, we reviewed several renal disease databases, including the United States Renal Data Systems and New York State End Stage Renal Disease Network. We have previously reported our experience with HIVAN at Mount Sinai Medical Center.

Results: The exact cause of the renal disease remains unknown. The importance of a direct effect of HIV-1 viral protein(s) or renal HIV-1 gene expression in disease pathogenesis is supported in the murine model of HIVAN, but definitive proof of renal cell infection in humans is lacking. Further study is required to clarify this issue. We estimate that HIVAN is the fourth leading cause of end-stage renal disease (ESRD) among Blacks between the ages of 20 and 64 years, only slightly behind hypertension, diabetes, and chronic glomerulonephritis. At Mount Sinai Hospital HIVAN accounts for 20% of newly diagnosed ESRD in young black adults. It has become the third leading cause of ESRD in this group, after hypertension and diabetes.

Conclusions: In seropositive patients with renal disease, renal biopsies should be performed to confirm the diagnosis and determine the true incidence. Special attention should be directed toward understanding the underlying cause(s) of HIVAN. A multicenter trial to explore the potential for antiviral therapy in this disease should be initiated. **Key Words:** HIV-1, chronic renal failure, acute renal failure, glomerulosclerosis, epidemiology, end-stage renal disease

PATIENTS WITH HIV-1 INFECTION are at risk to develop renal diseases with diverse etiologies. Acute renal failure occurs in up to 20% of hospitalized patients with HIV-1 infection (1), an incidence significantly higher than that seen in the general hospital population (2). Drug-induced nephrotoxicity and renal ischemia are common precipitating events, and it is possible that HIV-associated tubule defects can predispose patients to intravascular volume depletion and acute renal failure. The single most common cause of chronic renal insufficiency in HIV-1+ patients is HIV-associated nephropathy (HIVAN). This was first reported in 1984 by Rao and associates (3) who described focal segmental glomerulosclerosis and nephrotic-range proteinuria in a group of patients with clinically evident AIDS. The glomerular filtration rates of these patients declined rapidly, terminating in end-stage renal failure within 8 to 16 weeks. Mortality was extremely high, approaching 100% within 6 months of diagnosis. It is now recognized that this renal lesion can occur at any time in the course of HIV-1 infection, and the term "HIV-associated nephropathy" has replaced "AIDS nephropathy." Typical morphologic

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Address correspondence to Jonathan Winston, M.D., Assistant Professor of Medicine, Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1243, New York, NY 10029.

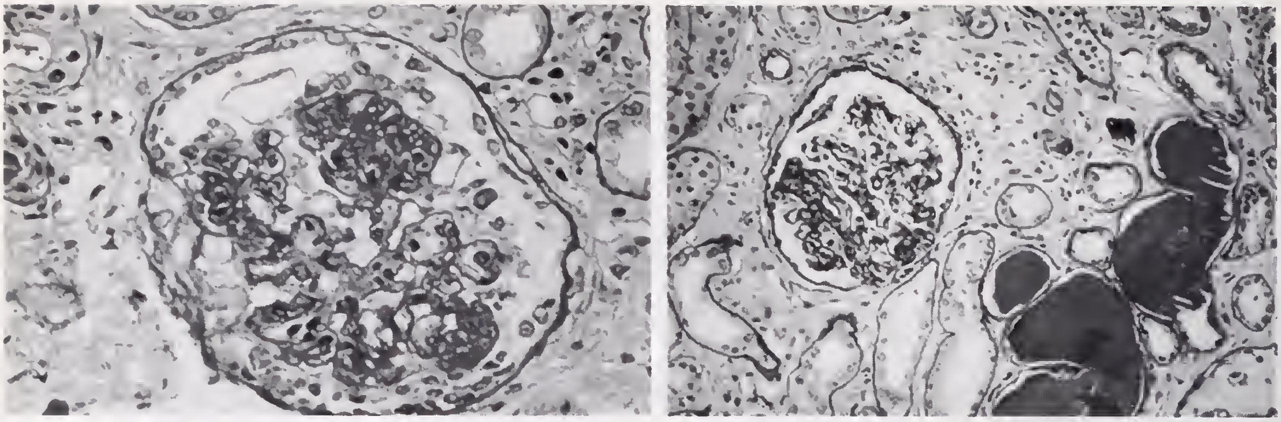

Fig. 1. Histopathology of human HIVAN. The glomerulus on the left shows segmental glomerular sclerosis with reactive glomerular epithelial cells. The figure on the right shows dilated tubules occupied by proteinaceous casts and surrounded by interstitial fibrosis.

features include enlarged kidneys, microcystic tubule dilation, tubulointerstitial inflammation, interstitial edema, and fibrosis. Glomerular involvement is usually focal and segmental with glomerulosclerosis, collapse of the glomerular tuft, and hypertrophy of visceral epithelial cells. Endothelial cells containing tubuloreticular inclusions are often reported but are a nonspecific finding (Fig. 1) (4).

Since 1984 more than 300 cases of chronic renal disease associated with HIV-1 infection have been described, and in at least 50% of cases, the clinical course and/or kidney biopsy has been consistent with the diagnosis of HIVAN (3, 5–16). However, when the renal biopsy in these HIV+ patients with chronic renal disease is not diagnostic of HIVAN mesangial hyperplasia is seen most often. The significance of this lesion is controversial. Some investigators have proposed that mesangial hypercellularity may be a precursor to focal glomerulosclerosis and have ascribed both lesions to a histopathologic continuum in HIVAN (17). One form of proliferative glomerulonephritis, which has been described with increasing frequency in HIV+ patients, is membranoproliferative glomerulonephritis, with or without coinfection with Hepatitis C (18, 19). Similar to HIVAN, this lesion can progress rapidly to renal failure. Several HIV+ patients have been described with an acute proliferative, crescentic glomerulonephritis of the IgA type with a polyclonal gammopathy, circulating immune complexes, and cryoglobulinemia (20–22). We and others have uncovered diabetic nephropathy and amyloid kidney disease in HIV+ patients suspected of having HIVAN (5, 9, 19, 23, 24). Therefore, a kidney biopsy is necessary to confirm the diagnosis because it is often impossible to distinguish HIVAN from other forms of glomerular involvement on clinical grounds alone.

Clinical Course

Although the syndrome of HIVAN is now recognized more frequently, our understanding of its natural history is incomplete. Because the diagnosis must be confirmed by kidney biopsy, clinical studies have suffered from the lack of distinction between HIVAN and other forms of chronic kidney disease. The rate of progression to renal failure tends to be rapid, with a median time of 10 weeks from initial evaluation to dialysis (11). Some reports indicate that a more indolent clinical course can be expected, especially when the disease is recognized in its early stage before the onset of azotemia or nephrotic-range proteinuria (25, 26).

Etiology of HIVAN

The exact cause of the renal disease remains unknown. The etiologic role for HIV-1 in HIVAN was established by the observation that transgenic mice expressing HIV-1 envelope and regulatory proteins develop a renal phenotype that is essentially identical with the disease in humans (27). The importance of a direct effect of HIV-1 viral protein(s) or renal HIV-1 gene expression in disease pathogenesis is now further supported by the observation that HIV-1 transgene expression induces renal epithelial cell death in transgenic kidneys grown *ex vivo,* and, transgenic kidneys develop renal disease even when transplanted into normal mice (28). The contention that direct renal infection is a cause of HIVAN is supported by other animal models of nephropathy such as FIV-infected cats (29).

In humans, definitive proof of renal cell infection is lacking (24, 30, 31, 32). Renal cells can be infected with HIV-1 *in vitro,* and *in situ* hybridization and polymerase chain reaction (PCR) of HIV DNA from microdissected renal tissue suggests the possibility of direct infection, but these observations are not specific for HIVAN. Studies reported this year suggest the possibility of a renal-tropic HIV strain, but such a strain has not been purified, cloned or shown to have true tropism (33).

HIV infection of renal epithelial cells cannot entirely explain all features of this disease, however, and genetic cofactors are clearly critical. Almost 90% of the cases of HIVAN occur in Blacks with the renal disease observed only rarely in Whites (7, 10, 34, 35). Blacks are also disproportionately affected by the HIV epidemic and are generally at greater risk for the development of renal disease. The new acquisition rate of AIDS in black women is nearly 15 times that of white women, and in black men, the new acquisition rate is 5-fold greater than for Whites. The definition of the second receptor for viral entry and the absence of mutation for these receptors in Blacks has given great insight into the potential genetic cofactors (36, 37). Viral tropism and/or the presence of a unique renal receptor could explain the racial predilection for HIVAN in humans.

Epidemiology of HIVAN

The renal disease should be viewed in the context of the overall HIV epidemic. Approximately 850 thousand to 1.2 million people are infected with HIV-1 in this country. The World Health Organization estimates that 20 million people currently have HIV infections worldwide (38–40). Initially, the epidemic was confined to homosexual and bisexual men. In recent years, however, the epidemic has changed quite dramatically. In areas of high prevalence, the ratio of male to female cases has fallen from 8:1 to under 2:1. The major reason for this change is the increase in heterosexual transmission. Homosexual and bisexual men continue to be major groups at risk with prevalence rates ranging from 20 to 70%, but the prevalence of HIV-1 infection in intravenous drug users is only slightly less (41–43). The prevalence of HIVAN nationally is population-dependent and variable; the prevalence is relatively low when the population studied comprises predominantly white homosexuals, and relatively high when the population studied is predominantly black. There is little evidence for a stabilization of the HIV epidemic in this country. Current estimates are that the new case rate of HIV will increase by 20% annually. As a result, HIV infection and its sequelae will remain a major health care issue until a cure is found or transmission can be prevented effectively. We can expect to see increasing numbers of patients with HIV-related renal diseases.

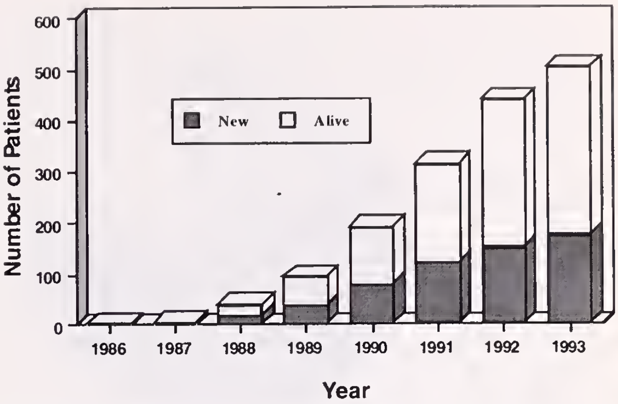

Fig. 2. Incidence of HIVAN in New York State [adapted from Neff and Rasmussen, (45)]. A 30% to 50% annual increase has occurred since 1989. (Reprinted with permission from J Am Soc Nephrol 1996; 7:1–7.)

As a cause of kidney failure, HIVAN is increasing in incidence, but because of concerns about patient confidentiality and underreporting, we do not have a reliable estimate of the true incidence. Despite the potential for errors, the data from the United States Renal Data System (USRDS) demonstrate that the incidence of HIVAN as a cause of kidney failure has more than tripled since 1990. For Blacks aged 20 to 65 years, HIVAN was the sixth leading cause of ESRD, nearly as common as lupus nephritis (44). These data likely underestimated the magnitude of the problem because of poor tracking of the diagnosis nationally.

In New York State, unlike the national registries, HIVAN has been closely tracked by ESRD Network #2 since 1986 (Fig. 2). Between 1989 and 1992, 387 new cases were reported in New York alone (45).

As of 1993, approximately 2,800 ESRD patients were HIV+ nationally (46). If, as a conservative estimate, 50% of these individuals have HIVAN and the median survival of HIV+ patients on dialysis is 12 months, then 700 patients with HIVAN are enrolling into the ESRD program each year. If these estimates are correct, HIVAN is the fourth leading cause of ESRD among Blacks between the ages of 20 to 64 years, only slightly behind hypertension, diabetes, and chronic glomerulonephritis. At The Mount Sinai Hospital in

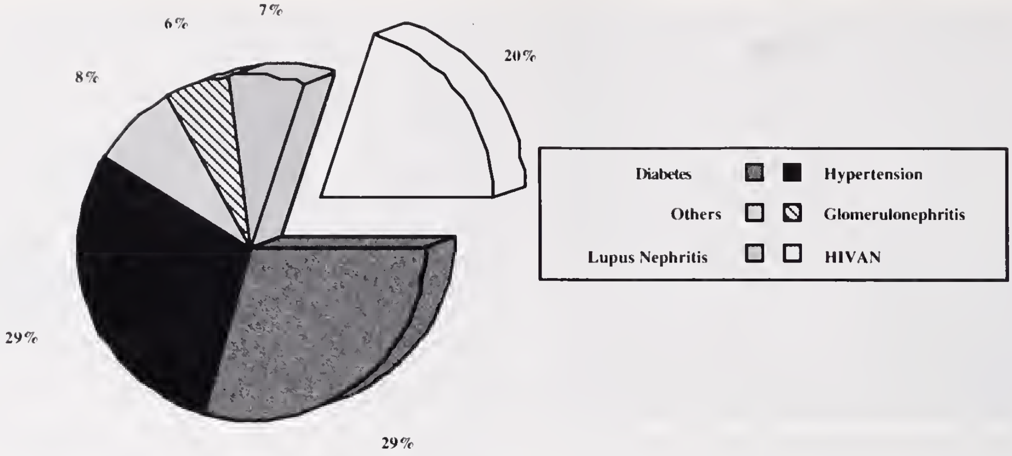

Fig. 3. Primary disease as a cause of end-stage renal disease in Blacks, age 20 to 64 years of age, The Mount Sinai Hospital, New York, 1992–1994.

New York, from 1992 to 1994, 8% of the 325 new patients entering our chronic dialysis program had HIVAN diagnosed either by biopsy or were HIV seropositive in association with rapid progression of renal failure with heavy proteinuria. In young black adults, HIVAN accounted for 20% of newly diagnosed ESRD. It has become the third leading cause of ESRD in this group, after hypertension and diabetes (Fig. 3) (47).

Treatment

An effective strategy for therapy depends on the pathogenesis of the disease. Current approaches include therapies that are not directed to the virus *per se* such as corticosteroids, other immunosuppressants, and converting enzyme inhibition (24, 48, 49). Strategies directed at the virus more specifically with antivirals are unproven but would likely be effective if viral replication were a component in the pathogenesis of HIVAN. In preliminary studies of HIVAN patients, viral burden was found to be surprisingly low in HIV+ patients with HIVAN before dialysis as contrasted to HIV+ patients with ESRD but without HIVAN. This suggests that viral burden may not be high in HIVAN and that another factor, such as viral tropism or a receptor variation, might be responsible. When HIVAN patients are placed on dialysis, however, viral burden appears to increase, thus these patients may be particularly responsible to antiviral therapy (23).

Summary and Recommendations

1. We must develop a national registry to track the incidence of HIVAN as a cause of ESRD, separate from other diagnostic categories. We must follow its natural history once dialysis is initiated.
2. Renal biopsies should be performed to confirm the clinical suspicion because the true incidence is unknown and the diagnosis affects prognosis.
3. Special attention should be directed toward the underlying cause(s) of HIVAN and the cofactor(s) which determine the predilection of this disease for Blacks.
4. A multicenter trial should be initiated to explore the potential for antiviral therapy in this disease and for patients undergoing dialysis.

Acknowledgment

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Cardiac Tamponade in a Patient with AIDS:

A Review of Pericardial Disease in Patients with HIV Infection

LOIS ESTOK, M.D., AND FRANCES WALLACH, M.D.

Abstract

Pericarditis in patients with human immunodeficiency virus (HIV) infection may be asymptomatic or symptomatic, but is sometimes overlooked because of signs and symptoms of other organ system diseases. A case report of cardiac tamponade in a patient with acquired immunodeficiency syndrome (AIDS) is presented. To determine the incidence and causes of pericardial disease in patients with HIV infection, a review of the literature was conducted. Fifteen autopsy and echocardiographic series involving 1139 patients with HIV infection reveal that the average incidence of pericardial disease is 21%. Most cases are asymptomatic and without an identifiable cause. However, in those that are symptomatic, about two thirds are caused by infection or neoplasm; one third have an undetermined etiology. In the 66 published cases of cardiac tamponade in patients with HIV infection, 26% are caused by tuberculosis, 17% are purulent, and 8% are caused by *Mycobacterium avium-intracellulare* (MAI). Lymphoma and Kaposi's sarcoma are each responsible for 5% of the effusions. Less frequent causes of tamponade are *Cryptococcus neoformans,* cytomegalovirus, and *Mycobacterium kansasii.* Small asymptomatic pericardial effusions in patients with HIV infection do not require diagnostic evaluation. However, large symptomatic pericardial effusions should be investigated, because two thirds are caused by potentially treatable infections or neoplasms. **Key Words:** human immunodeficiency virus, HIV, acquired immunodeficiency syndrome, AIDS, pericarditis, pericardial, cardiac tamponade, *Mycobacterium avium-intracellulare*

PERICARDIAL DISEASE in patients with HIV infection is common, but is usually asymptomatic. In patients with HIV infection pericardial effusion may be caused by opportunistic infections, tumor, metabolic or hemodynamic abnormalities, or it may not have an identifiable cause. We report a case of cardiac tamponade in a patient with advanced AIDS, followed by a review of the literature of pericardial disease in patients with HIV infection.

Case Report

A 42-year-old African American woman was admitted to Mount Sinai Medical Center in March 1995, when she showed signs of acute cardiac tamponade. She had received a diagnosis of HIV infection in November 1993 when she presented with *Pneumocystis carinii* pneumonia (PCP). She had refused antiretroviral therapy and was noncompliant with trimethoprim-sulfamethoxazole for PCP prophylaxis. She was well until January 1995 when she complained of dyspnea on exertion without fever or cough. Her vital signs and the results of her physical examination were normal except for a pulse rate at rest of 130 beats/min. The chest x-ray was normal. The electrocardiogram showed sinus tachycardia, nonspecific ST-T wave changes in the inferior and lateral

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From the Department of Medicine, Division of Infectious Diseases, Mount Sinai School of Medicine, New York. Address correspondence to Dr. Lois Estok, St. Mary Hospital, Department of Family Practice, 308 Willow Ave., Hoboken, NJ 07030.

leads, and minimal voltage criteria for left ventricular hypertrophy. Hemoglobin level was 10 g/dL and hematocrit value was 30%. Arterial blood gases on room air showed a pH of 7.44, pCO_2 of 29 mm Hg, pO_2 of 80 mm Hg, HCO_3 of 19.9 mEq/L, and O_2 saturation of 96.4%. The CD4 count was 20/mm^3 (normal range, 600–1200/mm^3). The results of the gallium scan and thyroid function tests were normal. The patient's tachycardia resolved spontaneously within 2 days.

She was readmitted in February 1995 for recurrent dyspnea on exertion. She had paroxysmal nocturnal dyspnea and a 13-pound weight loss during the preceding 2 months. Her examination was unremarkable except for a rapid pulse rate and decreased breath sounds at the right base. Her stool was negative for occult blood. Chest x-ray (Fig. 1) showed normal heart size and increased markings at the right base with blunting of the right costophrenic angle. Electrocardiogram showed sinus tachycardia at a rate of 145 beats/min and was unchanged from the prior month's electrocardiogram. She was anemic with a hemoglobin level of 6.6 g/dL for which she received 3 units of packed red cells. The ventilation-perfusion lung scan and gallium scan were normal. The urine toxicology screen was positive for cocaine. Her tachycardia resolved within 2 days. It was felt that her tachycardia was secondary to cocaine-induced cardiomyopathy and anemia. A bone marrow biopsy was performed which revealed rare acid-fast bacilli (AFB) and a poorly formed granuloma. She declined antimycobacterial medications.

In March 1995, she reported increasing dyspnea for several weeks and marked weakness. She denied using crack cocaine since her last admission. On physical examination she was afebrile and had a weak, thready pulse, blood pressure of 96/70 mm Hg, pulsus paradoxus, no rub, II/VI systolic murmur, normal heart sounds, and clear lungs. Chest x-ray (Fig. 2) showed gross cardiac enlargement with minimal blunting of the costophrenic angles. The electrocardiogram had not changed from the previous month's tracing. Kidney and liver function tests showed normal results. The serum total protein value was 5.3 g/dL and albumin level was 1.8 g/dL. The urine toxicology screen was negative for cocaine, opiates, benzodiazepines. The hemoglobin level was 8.8 g/dL, the white blood cell count was 1900/mm, and the platelet count 187,000/mm^3. An emergent echocardiogram (ECHO) showed a large anterior and posterior pericardial effusion, four-chamber dilatation with severe biventricular dysfunction, moderate mitral regurgitation, mild aortic, pulmonic and tricuspid regurgitation, and partial right ventricular collapse with impending tamponade. Left ventricular stroke volume manifested a 20% variation. An ECHO-guided pericardiocentesis yielded only 30 cc of serous loculated fluid. A repeat attempt to remove more pericar-

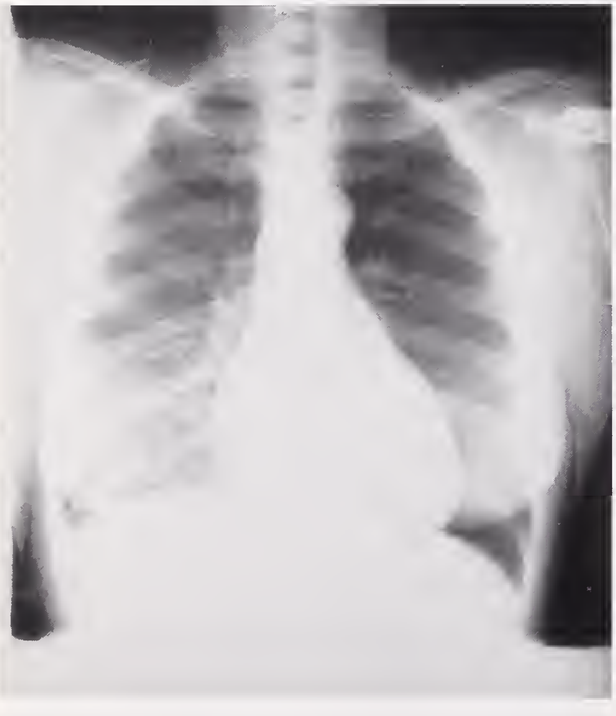

Fig. 1. Posteroanterior x-ray film of the chest, 2/10/95, showing a normal size heart.

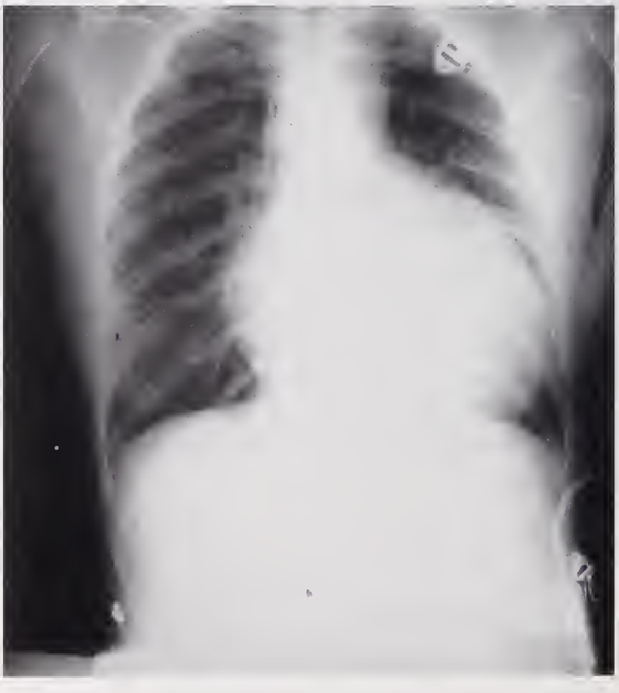

Fig. 2. Posteroanterior x-ray film of the chest, 3/9/95, showing gross cardiac enlargement.

dial fluid at the bedside was unsuccessful, because the patient suddenly sat up during the procedure. The right ventricle was pierced, causing ventricular fibrillation. She was immediately resuscitated. An emergency subxyphoid pericardial window was performed, and 600 cc of bloody fluid was drained. The pericardium appeared normal at surgery. The total protein of the pericardial fluid was 5.8 g and the glucose level was 103. There were no white blood cells or organisms seen on Gram stain. AFB stain, stains for the presence of fungi in the fluid, and culture of the fluid for bacteria, including mycobacteria, and fungi were negative. The pericardial biopsy showed a dense connective tissue membrane and was negative for granulomas, chronic inflammation, or tumor. Special stains for AFB and fungus were negative. The patient was treated with isoniazid, rifampin, ethambutol, ciprofloxacin to cover *Mycobacterium tuberculosis/Mycobacterium avium-intracellulare.* She had severe nausea with clarithromycin and pyrazinamide. She initially improved on antimycobacterial medications and diuretics for what was believed to be HIV-associated cardiomyopathy. The culture of her bone marrow biopsy, pericardium, and induced sputum eventually grew out MAI. Isoniazid, rifampin, and ciprofloxacin were stopped, and rifabutin and clofazimine were added to ethambutol.

In April 1995, her heart size was normal on the chest x-ray and an echocardiogram showed resolution of her pericardial effusion, but persistent severe left ventricular dysfunction. She was noncompliant with her medications. In May 1995 her chest x-ray showed recurrence of a globular heart and clear lung fields. In June 1995 she was admitted for a major upper gastrointestinal bleed secondary to a 3.4-cm gastric ulcer. Biopsy of the lesser curvature ulcer was negative for opportunistic infections and tumor. Chest x-ray in June 1995 was unchanged from the previous x-ray. Abdominal computed tomographic (CT) scan with and without contrast noted a pericardial tomographic (CT) scan with and without contrast noted a pericardial effusion and multiple low attenuation lesions in the liver, a normal spleen, diffusely enlarged pancreas, and diffuse mesenteric adenopathy. Blood culture from May 1995 and a stool culture from June 1995 grew out MAI. In July 1995 she was admitted for severe diarrhea, dehydration, and hypotension. Chest x-ray showed a normal sized heart and clear lung fields. In accord with her wishes, she was treated only to make her more comfortable. She died 5 days after admission. Permission for autopsy was denied. It was believed that she died of disseminated MAI and HIV-associated cardiomyopathy.

Methods

We conducted a literature search of the Medline databases from 1982 to June 1996. Medline is produced by the National Library of Medicine and includes more than 3800 biomedical journals. Medical subject heading search terms included the following: acquired immunodeficiency syndrome, AIDS, human immunodeficiency virus, HIV, pericarditis, pericardial, and cardiac tamponade. Only English language journals from North America and Europe were included.

Results

Incidence. The incidence of pericardial disease in patients with HIV infection in five autopsy series ranged from 9 to 32%, and in 10 echocardiographic series, from 8 to 53%. The average incidence of pericardial disease in the 15 series combined was 21% (1–15) (see Table 1). Most pericardial effusions had a modest volume and were not suspected clinically. Rarely did the pericardial effusions contribute to the patient's death. In one autopsy series of 115 patients with AIDS (11), 35 of 37 cases were serous and without evidence of infectious or neoplastic etiology, and often associated with serous pleural effusions or ascites. In an echocardiographic series of 102 consecutive, hospitalized patients with AIDS (4), 9 had small pericardial effusions, 30 had moderate pericardial effusions, and none had large effusions. In another echocardiographic series of 70 consecutive asymptomatic and symptomatic patients with HIV infection (12), 6 had small, silent pericardial effusions and one had a moderate-sized pericardial effusion with impending tamponade. This patient refused pericardiocentesis and responded significantly to treatment with a nonsteroidal anti-inflammatory drug. In another echocardiographic series of HIV-seropositive patients (14), 42% of pericardial effusions resolved without specific therapy. It is believed that pericardial effusions not associated with opportunistic infections, tumors, or obvious metabolic disturbances such as uremia, are a direct or indirect consequence of HIV infection.

Rate of Pericardial Disease vs Myocardial Disease. For comparison, the incidence of myocardial disease at 18% was slightly less than pericardial disease in patients with HIV infection at 21% (see Table 1). The most common myocardial abnormality at autopsy in patients with HIV infection was focal interstitial mononuclear infil-

TABLE 1
Incidence of Pericardial Effusion in Patients with HIV Infection

Author et al. (Ref.)	Year	Type of study	No. of Patients	No. with pericardial effusions	% with effusions	No. with cardiomyopathy	% with cardiomyopathy
Fink (1)	1984	Echo/Autopsy	15	8	53	3	20
Reitano (2)	1984	Echo	25	7	28	3	12
Roldan (3)	1987	Autopsy	54	5	9	17	31
Corallo (4)	1988	Echo	102	39	38	55	54
Klatt (5)	1988	Autopsy	187	33	18	17	9
Monsuez (6)	1988	Autopsy	86	18	21	13	15
Raffanti (7)	1988	Echo	12	1	8	2	17
Hecht (8)	1988	Echo	27	7	26	8	30
Anderson[a] (9)	1988	Autopsy	71	15	21	37	52
Levy (10)	1989	Echo	60	9	15	14	23
Lewis[b] (11)	1989	Autopsy	115	37	32	2	2
Himelman (12)	1989	Echo	70	7	10	8	11
Steffen (13)	1991	Echo	151	29	19	6	4
Blanchard (14)	1991	Echo	50	11	22	7	14
De Castro (15)	1992	Echo	114	13	18	12	17
Total			1139	239	21	204	18

[a] Includes 58 patients reported previously by Reilly et al. in 1988 (16).
[b] Includes 41 patients reported previously by Cammarosano et al. in 1985 (17).

trates, with or without myocyte necrosis or fibrosis (9). HIV has rarely been cultured from myocardium (18). It is believed that the myopathy is either caused by a direct toxic effect of the virus or indirect effects of HIV infection through other mediators, such as the cytokine cascade (19). Occasionally, opportunistic pathogens have been found in the myocardium at autopsy.

Symptomatic Pericardial Disease. The overall incidence of clinically apparent heart disease in one series of 300 AIDS patients (6) was only 5%. Of those with clinical heart disease, pericardial disease was the most common abnormality: 22% had echocardiographic evidence of tamponade, 33% had large pericardial effusions, and 17% had moderate pericardial effusions (total, 72%). Thirty-three per cent had dysynergy with or without dilated left ventricle on M-mode or two-dimensional echocardiogram. In another series of 25 patients with AIDS-related complex (ARC)/AIDS and pericardial effusions (20), 32% presented with tamponade. Patients with advanced HIV infection were more likely to have underlying cardiac disease than those with less advanced HIV infection. Patients with HIV infection who have cardiac disease had a mean CD4 of 143 ± 92/mm^3, compared with those without cardiac disease, who had a mean CD4 of 294 ± 243/mm^3 (6). As opposed to clinically silent pericardial effusions, symptomatic pericardial effusions (friction rub, substernal chest pain, fever, tamponade), were usually caused by pericardial infection or neoplasm. There were 66 cases of cardiac tamponade in the English literature in patients with AIDS in the United States and Europe (1, 15, 20–41), (see Table 2). In 37 of the 66 cases, the pericardiocentesis fluids were described. All but two were described as exudative, serosanguinous, or purulent. Of the two that were described as straw-colored, one was because of MAI and one because of cytomegalovirus (CMV) infection. Of the 66 reported cases of cardiac tamponade, 26% were caused by tuberculosis, 17% were purulent, 8% were caused by MAI, 5% by lymphoma, 5% by Kaposi's sarcoma, and 3% by CMV. The cause could not be identified in 32% of the cases (see Table 2). There were also reports in the literature of large pericardial effusions in patients with HIV infections which did not cause tamponade. Additional etiologies noted in those patients included herpes simplex infections (type I and type II) (37, 38), Nocardiosis, (39), toxoplasmosis (6, 45), *Salmonella typhimurium* endocarditis (6), and *Listeria monocytogenes* (48). In three case series with a total of 31 patients (31, 32, 34), large pericardial effusions with or without tamponade in patients with AIDS were the result of an identifiable infectious or neoplastic cause in 42% of the cases.

MAI Infection of the Pericardium and Myocardium. Our patient with advanced AIDS, HIV-associated cardiomyopathy, and cardiac tamponade due to MAI was very similar to a case reported by Cohen et al. in 1986 (21). A pericardial biopsy was not done, their patient with AIDS who had a dilated cardiomyopathy and cardiac tamponade, but the serosanguinous pericardio-

TABLE 2

Causes of Cardiac Tamponade in 66 Patients with HIV Infection

Author et al. (Ref)	*M. tuberculosis*	MAI	*M. kansasii*	Purulent	Cryptococcus	CMV	Lymphoma	Kaposi's sarcoma	Unknown
Fink (1)									3
Schuster (21)					1				
Cohen (22)		1							
Sunderman (23)	1								
Stechel (24)				1[a]					
Brivet (25)					1				
Steigman (26)								1	
Stokla (27)								1	
Helfand (28)							1		
Scott (29)						1			
Turco (30)		1		1			1		
Nathan (31)						1			
Karve (32)				2[b]					
Reynolds (33)	6[c]	2							2
Eisenberg (20)	1[c]								1
Remick (34)								1	
Galli (35)									8
Horn (36)	2								
Kwan (37)	4[c]			3[a]					7
Grant (38)				1[a]					
De Castro (15)				1[a]					
Decker (39)				2					
Moreno (40)			2						
Scerpella (41)				1					
Serrano (42)	3								
VanVooren (43)							1		
Estok		1							
Total number	17	5	2	11	2	2	3	3	21
% of cases	26	8	3	17	3	3	5	5	32

[a] *Staphylococcus aureus.*

[b] *Streptococcus pneumoniae.*

[c] Documented or presumed *M. tuberculosis* because pulmonary or miliary tuberculosis was present.

centesis fluid grew MAI. Our patient's pericardial fluid initially was clear. During the second procedure of creating a pericardial window, the fluid was described as serosanguinous. However, the blood in the fluid was believed to be secondary to previous trauma to the myocardium by the pericardiocentesis needle.

There are five other cases in the English literature documenting MAI in the myocardium or pericardium in patients with AIDS who have cardiac tamponade (6, 9, 30, 49, 50). The first paper to report a cardiac finding in a patient with AIDS was by Autran et al. in 1983 (49). They described Kaposi's sarcoma on the whole anterior wall of the heart of a Haitian woman who died of febrile cardiogenic shock. On histology there were also periodic acid-Schiff (PAS) staining, Gram-positive bacilli in macrophages, which suggested Whipple's Disease. However, in retrospect, these bacilli probably represented MAI. Another case report (50) described a patient with AIDS who developed substernal chest pain, fever, and friction rub. Pericardiocentesis yielded 220 cc of serosanguinous fluid. The stain for AFB was negative, but MAI was cultured from the fluid. After 2 months of antimycobacterial therapy the patient died. At autopsy the myocardium appeared pale, and the ventricles were enlarged. The pericardium was thickened with dense fibrous adhesions. On histology, the myocardium was normal except for interstitial edema. Both surfaces of the pericardium showed numerous AFB-laden histiocytes. In another case of cardiac tamponade in a patient with AIDS who had disseminated MAI, CMV retinitis, and cardiomegaly with anasarca, the pericardiocentesis yielded much straw-colored fluid which grew MAI (30). These case reports suggest that MAI bacteremia can seed the myocardium and pericardium, in which MAI may proliferate and form AFB-laden histiocytic infiltrates.

Management. Pericardial effusions in HIV-seropositive patients, in the absence of signs or symptoms of inflammation or hemodynamic compromise, usually do not require pericardiocentesis. Most of these are idiopathic and remain asymptomatic, and may even remit spontaneously. These clinically silent pericardial effusions are believed to be related to underlying metabolic or hemodynamic disturbances. However, in symptomatic pericardial effusions, an identifiable underlying infectious or neoplastic cause may be found in about half of the patients. HIV-seropositive patients from the inner city who have pulmonary infiltrates and fever are especially at risk for tuberculosis as the cause of their symptomatic pericardial effusion (33). Pericardiocentesis may not only be diagnostic, but also therapeutic in the cases of tamponade. Pericardial biopsy may increase the diagnostic yield. An open drainage procedure, usually done with the subxyphoid approach, may be necessary for large effusions. Open drainage is required for purulent pericarditis. Further management of pericardial effusions caused by infections or neoplasms in HIV-seropositive patients is similar to that in HIV-seronegative patients. Large idiopathic pericardial effusions may respond to nonsteroidal anti-inflammatory drugs (12).

Summary

In summary, pericardial disease is the most common cardiac finding in HIV-seropositive patients. It affects approximately one fifth of these patients. Pericardial effusions can range from small asymptomatic effusions which are caused by direct or indirect effects of HIV infection, to life-threatening cardiac tamponade. The underlying etiologies of the cardiac tamponade include infections and neoplasms. Tuberculosis, purulent pericarditis, and disseminated MAI comprise approximately 50% of all the confirmed causes. Kaposi's sarcoma and lymphoma are the two most common neoplasms producing pericardial effusions in patients with AIDS but account for a much smaller percentage of the cases. About one third of the cases of cardiac tamponade are without an identifiable cause. Treatment is similar to that for patients who do not have AIDS.

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Dermatology Notes

Risks of HIV Infection

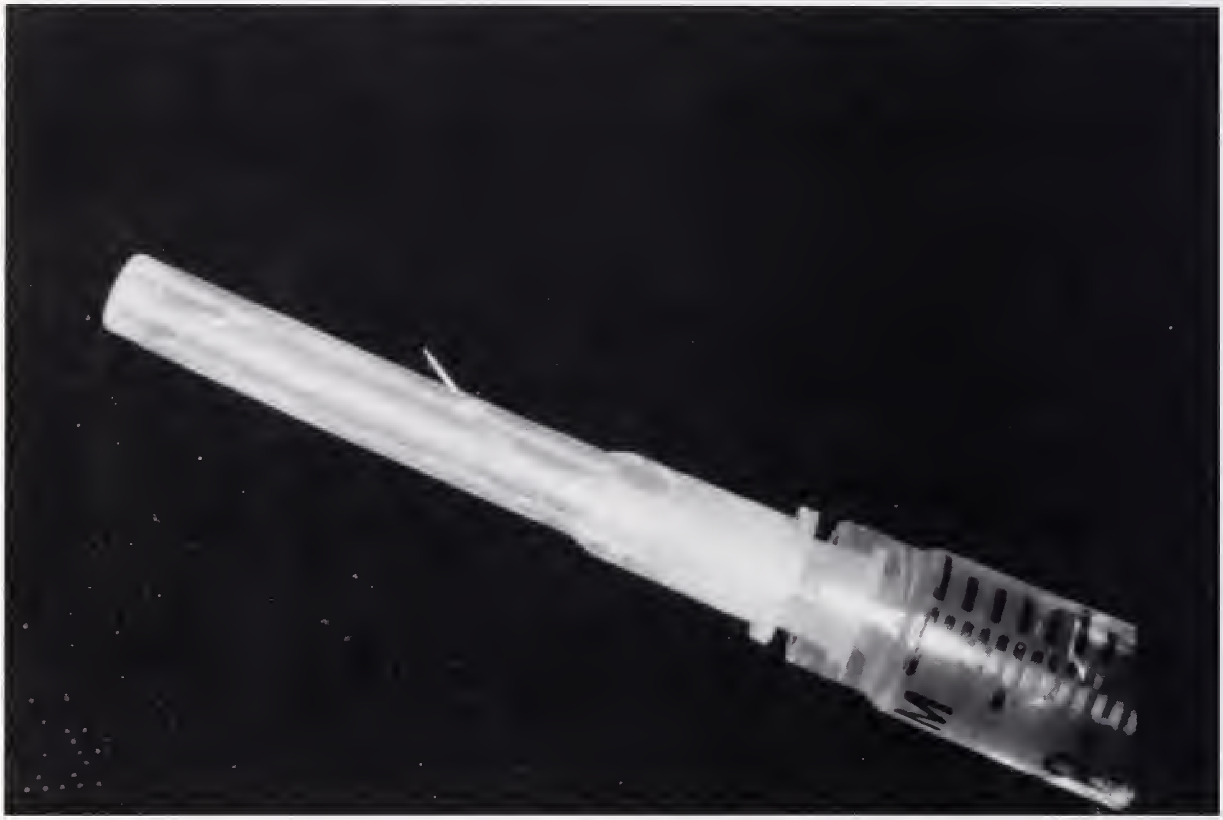

Fig. 1. Recapping of syringes has proven to be one of the most common sources of needle stick injuries that put workers at risk of exposure to HIV infection. A syringe needle is shown penetrating its cap.—*Ron Shelton, M.D., and Mark Lebwohl, M.D.*

Editorial

SHERMAN KUPFER, M.D.

Medical Editors Trial Accounting (META)

I AM PLEASED TO INFORM the readers that *The Journal* has joined with an international cohort of esteemed medical journals to promote future developments in medical publications, whether they be paper or electronic. Foremost concern has been directed at the question of the necessity and quality of peer reviews. *The Journal* believes that peer review is essential, and strives to assure that the comments and critiques offered to the authors by its ever changing, but always dedicated referees are useful and constructive irrespective of the editorial decision to accept or reject a particular manuscript. The pride of authorship is highly regarded by *The Journal,* but the interests of the readers remain paramount. It is the goal of the group of medical editors and scholars who have joined together to form the World Association of Medical Editors to promote these aims. There are many issues and new approaches to be addressed.

A recent program has been launched by this group to enhance the availability of results of controlled clinical trials, many of which never seem to be reported. The program has been labeled the "Medical Editors Trial Amnesty." I am not sure that I agree with the use of the word amnesty in the title, because I do not think that editors can pardon a heretofore failure to report. I have preferred to label the effort to obtain potentially very useful information as an opportunity to trialists to take account of their results and report them so that the overall experience can be tabulated in the best possible way. My reasoning is outlined in the following presentation, much of which had been prepared by the META group. With their permission, I have made several changes, as an editor is wont to do.

Medical Editors Trial Accounting (META)

Reports of properly conducted, randomized controlled trials are the foundation of safe and effective health care. However, a substantial proportion of all controlled trials never contributed to this knowledge base because they are not submitted for publication (1, 2). This fact has several important implications for patient care. First, underreporting of trials reduces the power of systematic reviews to detect moderate but clinically important treatment effects. As a result, patients may be denied effective forms of health care. Second, because trials that show more promising treatment effects are more likely to be submitted for publication, research syntheses based on published studies can give misleading conclusions about treatment effectiveness, thus exposing patients to useless or even harmful therapies (3). Finally, patients may be asked to participate in new research studies designed to address questions that have, in truth, already been answered (4).

Trials go unreported for a myriad of reasons. The most common is that investigators think the results are "not interesting." It is well documented that trials with nonsignificant results are less likely to be submitted for publication (1). Sometimes, recruitment of participants takes longer than anticipated at the expense of time and resources set aside for report writing; investigators may relocate with the result that important work may remain unfinished; or investigators may discover a recently published trial on the same topic and conclude that their own results are now redundant. Editors must also take some of the responsibility when, by virtue of limitation in space, they are forced to decline to publish reports. Many investigators regret not having published their trial results, and almost all investigators, when contacted about unreported data, are delighted to provide it.

Because of the important consequences of unreported trials, the editors of nearly 100 international medical journals have joined together to call for an accounting and registration of any as yet unreported trial. I hope that investigators will see this as an opportunity to make the results of previously unreported trials publicly accessible. This effort potentially could contribute to the scientific foundation of health care. All investigators with unreported trial data are urged to register their trial(s) by filling in and returning an “Unreported Trial Registration Form” a copy of which can be found on the inside of the back cover. Particular attention should be given to those trials in which the results may have been presented or published as an abstract. Registration can be undertaken by anyone able to provide the registration information, even if they are unable to provide the actual trial data. The possibility of duplicate registration should not deter investigators or individuals from completing and submitting the information in their possession. Registration information can be posted or faxed to the editorial office of *The Journal*. Alternatively, registration information can be sent by e-mail (meta@ucl.ac.uk). The information will be made available by listing trial details on a web site, and in other ways as appropriate. If specific trial data are required, for example by those conducting systematic reviews, then the reviewer(s) will be able to seek this information directly from the trialist. Some of the trials may be suitable for full publication, and *The Journal* will be happy to consider these.

Medical editors are acutely aware of the trials and tribulations of research reporting. But on this occasion, because of the serious implications of unreported research, every effort is being made to cleave the trials from the tribulations. There is no confident estimate of the total number of unreported trials, but I hope that this constructive effort will be productive and enjoy a useful outcome.

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Abstracts

*The following abstracts were presented by investigators at the Fifteenth Annual Research Day at Mount Sinai School of Medicine. Most of the investigators serve in the Samuel Bronfman Department of Medicine including those working at affiliated institutions such as the Bronx Veterans Affairs Medical Center, Bronx, NY, Elmhurst Hospital Center, Elmhurst, NY, Englewood Hospital and Medical Center, NJ, Queens Hospital Center, Queens, NY, Saint Barnabas Medical Center, NJ, Newark Beth Israel Medical Center, NJ, and St. Joseph's Hospital and Medical Center, NJ. Four abstracts identified with an * were awarded special prizes and two abstracts identified with an † were selected for oral presentation on Research Day. Several additional reports were presented or shown, but the abstracts have been published elsewhere.*

Research in Medicine 1997
Mount Sinai School of Medicine

Alcohol Research

†Blood Alcohol Levels Are Increased in Social Drinkers Receiving Ranitidine. S. Arora, E. Baraona, and C. S. Lieber. Alcohol Research Center, Bronx VA Medical Center and Mount Sinai School of Medicine, New York, NY.

Ranitidine increases blood alcohol concentrations (BAC) by decreasing the first-pass metabolism (FPM) of ethanol. The effect of ranitidine on BAC has been found to be variable when using single large doses of alcohol or conditions (such as fasting or dilute alcohol solutions) in which FPM is minimal. By contrast, there is a general consensus that the drug increases BAC after postprandial consumption of a single small dose of ethanol (0.15 g/kg), but this effect has been considered clinically insignificant because the resulting BAC are low. However, social drinking comprises repetitive consumption of small doses. To study possible effects of ranitidine on such drinking, BAC was determined by breath analysis in 9 male social drinkers (<40 g alcohol/week) after four drinks of 0.15 g/kg ethanol given postprandially every 45 min, before and after ranitidine (150 mg b.i.d. for 7 days). Their BAC increased with repeated doses of ethanol, reaching a peak BAC of 24 ± 3 mg/dL before ranitidine and 33 ± 2 after ranitidine ($p < 0.05$), a change of an order of magnitude similar to that previously reported with cimetidine. Moreover, BAC remained greater than 25 mg/dL for a considerably longer time after ranitidine (54 ± 15 min) than before the drug (25 ± 12, $p < 0.05$). After ranitidine, seven of the nine subjects exceeded 25 mg/dL, a level at which impairment of judgment and finely tuned skills begins to occur and which exceeds the legal limits for driving in some European countries. With alcohol, eight of the nine subjects were clearly euphoric after ranitidine, whereas only three of them were euphoric before ranitidine. FPM was assessed from the difference between the quantity of alcohol imbibed and that reaching the blood before and after ranitidine. The quantity of alcohol reaching the blood was calculated by integration of the Michaelis-Menten equation over the entire blood alcohol curve. The deficit in absorption caused by FPM, which was 34 ± 6% before ranitidine, decreased to 12 ± 4% after ranitidine ($p < 0.001$), thereby increasing the bioavailability of alcohol.

In conclusion, under conditions mimicking social drinking, ranitidine increases BAC to levels known to impair the psychomotor skills required to perform complex tasks, such as driving. Moreover, it maintained those BAC during prolonged periods because of an increased bioavailability of alcohol. Thus, patients treated with ranitidine should be warned of the possibility that unexpected functional impairment may develop if they engage in social drinking.

Ethanol and Iron Diminish Hepatic Microsomal CYP2E1 Activity Whereas Polyenylphosphatidylcholine (PPC) Provides Full Protection. M. Aleynik, M. A. Leo, S. Aleynik, and C. S. Lieber. Alcohol Research Center, Bronx VA Medical Center and Mount Sinai School of Medicine, New York, NY.

Dietary iron overload decreases microsomal cytochrome P450 (CYP450) and damages membrane phospholipids. We wondered whether this might also pertain to CYP2E1, and whether it could be prevented by PPC (a 94–96% pure mixture of linoleate-rich polyunsaturated phosphatidylcholines) that protects against other liver injuries. For 8 weeks, 56 rats were fed our standard liquid diet with either PPC (3 g/1000 Cal) or equivalent amounts of linoleate (as safflower oil). Enzyme activities reflecting CYP2E1 were determined: the microsomal ethanol-oxidizing system (MEOS), assessed by the conversion of ethanol to acetaldehyde (measured by head space GC), and *p*-nitrophenolhydroxylase (PNP) activity, measured by high-performance liquid chromatography with UV detection of the product of the hydroxylation reaction, 4 -nitrocatechol. With ethanol (36% of energy) replacing carbohydrates, PNP activity increased from 0.27 ± 0.01 to 4.34 ± 0.13 nmol/min/mg protein; however, when carbonyl iron (5 g/1000 Cal) was added, the induction was 50% less ($p < 0.001$). Analogous effects were observed with MEOS ($p < 0.01$). PPC fully restored the enzyme activities that were reduced by iron: 4.73 ± 0.32 vs 2.73 ± 0.44 nmol/min/mg protein ($p < 0.001$) and 11.47 ± 0.79 vs 7.28 ± 1.09 ($p < 0.001$) for PNP and MEOS, respectively. The effects were similar whether expressed per milligram of microsomal protein or per nanomole of total P450. PPC is rich in linoleic acid but, when the latter was given as triglycerides (safflower oil), there was no protection. Total hepatic nonheme iron was the same in both groups. These observations indicate that the membrane protection provided by PPC includes the activity of some key enzymes such as CYP2E1, possibly by offsetting the pro-oxidant action of iron and ethanol.

In conclusion, induction of 2E1 by chronic ethanol consumption (determined by PNP and MEOS activities) is significantly decreased by iron, an effect that is fully prevented by PPC supplementation.

Polyenylphosphatidylcholine Opposes Alcohol-induced Oxidative Stress in Rat Pancreas. S. I. Aleynik, M. A. Leo, M. Aleynik, and C. S. Lieber. Alcohol Research Center, Bronx VA Medical Center and Mount Sinai School of Medicine, New York, NY.

In the pathogenesis of acute and chronic pancreatitis, alcohol abuse and subsequent oxidative stress plays a major role. We found recently that polyenylphosphatidylcholine (PPC), a 96% pure polyunsaturated phosphatidylcholine preparation extracted from soybeans, protects against oxidative injury in the liver of alcohol-fed baboons and CCl_4-treated rats. We wondered whether it might also be favorable in the pancreas. To that effect, for 2 months, 24 Sprague-Dawley rats were pair-fed ethanol (36% of energy) or isocaloric carbohydrate in our standard liquid diet supplemented with either PPC (3 g/L) or safflower oil (2.7 g/L) providing an equivalent amount of linoleic acid. 4-Hydroxynonenal (4-HNE) and esterified F_2-isoprostanes (F_2-IP), two specific parameters of *in vivo* lipid peroxidation, were extracted from the pancreas and measured by gas chromatography/mass spectroscopy. In the animals fed the safflower oil, alcohol doubled the 4-HNE (17.9 ± 3.3 vs 8.6 ± 1.1 ng/g; $p < 0.001$), whereas PPC totally protected against this oxidative stress (6.5 ± 0.6 ng/g; $p < 0.001$). Similar findings were obtained when the results were expressed per mg protein. F_2-IP were increased more than 50% by ethanol ($p < 0.01$) and PPC was again protective. There was a significant correlation between 4-HNE and F_2-IP. The protection against lipid peroxidation in the pancreas was as striking as that observed before in ethanol- or CCl_4-induced liver injury, indicating that the PPC effect is not organ specific. Dietary iron overload has been shown to increase lipid peroxidation and cause pancreatic atrophy. To assess the effect of iron on lipid peroxidation, another group of 32 rats received the same diet supplemented with carbonyl iron (5 g/L) for 2 months. Iron supplementation resulted in a 2-

to 3-fold increase in iron accumulation; but in the absence of ethanol, lipid peroxidation was unaffected.

The results indicate that: 1) alcohol produces an oxidative stress in the rat pancreas, as documented by significant and parallel increases of 4-HNE and F_2-IP; 2) PPC provides total protection. In view of the innocuousity of PPC, its clinical evaluation is now warranted not only in hepatic but also in extrahepatic disorders possibly due to oxidative stress, including pancreatitis.

Stimulation of Monocyte Interleukin-8 by Lipid Peroxidation Products: A Mechanism for Alcohol-induced Liver Injury. A. Jayatilleke and S. Shaw. Mount Sinai School of Medicine and Alcohol Research Center, Bronx VA Medical Center, New York, NY.

Interleukin-8 (IL-8), an inflammatory cytokine which promotes neutrophil accumulation, has been implicated in the pathogenesis of alcoholic liver disease, but the mechanism of its production is unknown. The ability of lipid peroxidation products, also implicated in alcoholic liver disease, to stimulate IL-8 production was studied because of their alleged role in alcoholic liver disease. IL-8 production by peripheral blood monocytes was measured using an IL-8 enzyme-linked immunoabsorbant assay (Biosource, Camarillo, CA). Human monocytes were isolated from EDTA-treated whole blood using a density gradient of Nycodenz. Incubations of monocytes were carried out in RPMI 1640 buffer for 18 hours, and IL-8 production was linear during that interval. In each experiment to verify both quantitation and function of monocytes, IL-8 production was determined with and without stimulation by lipopolysaccharide. Fatty acids as well as microsomes were peroxidized in *in vitro* incubations. Peroxidized fatty acids (arachidonic and linolenic) as well as microsomal membranes stimulated IL-8 production by peripheral blood monocytes whereas ethanol and acetaldehyde did not. Hydrocortisone (5 μg/ml), when incubated with monocytes along with peroxidized arachidonic or linolenic acid, suppressed IL-8 levels in comparison with the peroxidized arachidonic or linolenic acid alone, and kept production somewhat lower than basal production. The results obtained suggest that production of reactive oxygen radicals during ethanol metabolism may play a dual role in injury, primarily through direct injury of liver organelles, but secondarily by stimulating monocytes to produce IL-8, further causing liver injury through neutrophil chemotaxis.

Weight Change in Chronic Alcoholics in the Immediate Several Weeks of Abstinence: A Prospective Inpatient Detoxification- and Rehabilitation-Based Study. S. Mehta and S. Feinman. Alcohol Research Center, Bronx VA Medical Center and Mount Sinai School of Medicine, New York, NY.

The relationship between alcohol consumption and body weight has been the subject of considerable research and debate. In a study by Liu et al., the authors examined the relationship between alcohol intake and body weight in 7,230 US adults followed prospectively for 10 years. At baseline, women reporting at least one drink per day weighed less than nondrinkers. Prospectively, drinkers gained less weight than nondrinkers. Clevidence et al. demonstrated a difference in alcohol metabolism between lean and heavy women, with lean women requiring more energy to maintain weight. The present study was undertaken to assess the change in weight in actively drinking alcoholics admitted for inpatient detoxification and the factors influencing it. All patients admitted to the "detox" floor between December 1994 and April 1996 were included in the study. The patients had previously been stabilized on the medical floor. Patients with persistent medical problems, active psychiatric problems, AIDS, or durations of stay shorter than 14 days were excluded from the study. All patients had a careful history taken with emphasis on the amount, duration and date of last drink, smoking, and their usual weight. A careful physical examination with recording of their height and weight was conducted. All patients had a baseline complete blood cell count, prothrombin time, and liver profile. All patients were offered an *ad libitum* diet in consultation with the dietitian, and weight was recorded on a weekly basis. Smoking was discouraged but not prohibited. The mean change in weight at 4 weeks was calculated for the entire group and separately for those with a normal Body Mass Index (BMI), i.e. between 21 and 28 and for those above or below this range. It was also calculated for smokers vs nonsmokers.

During the study period, 170 patients were admitted. Of these, 28 were excluded because the duration of stay was less than 14 days. Another 4 patients were excluded because of advanced liver disease. Five patients with HIV infection but not AIDS were included in the final analysis. Thus there was a total of 138 patients: 41 smokers and 93 nonsmokers. Overall the patients gained 1.47 kg (SD = 2.90). Patients with a BMI of less than 21 (n = 24) gained an average of 3.02 kg (SD = 3.16), those with a BMI of 21 to 28 (n = 81) gained 1.32 kg (SD = 2.69), and those with a BMI greater than 28 (n = 33) gained 0.43 kg (SD = 2.895). Using ANOVA for comparing the groups revealed a highly significant difference among the three groups with a p value <0.006. Using the Neuman-Keuls test for pairwise comparisons of groups revealed a highly significant difference between those with a BMI of less than 21 compared with the other two groups ($p < 0.01$). There was no significant difference between those with a BMI of 21 to 28 and greater than 28. The regression equation was: Weight gain in kg = 9.85 − 0.33 × BMI (baseline). There was no difference between those who smoked and those who did not. This is the first report, of which we are aware, concerning the change in weight in chronic alcoholics with abstinence. Thin people tended to gain more weight on abstinence. This may be related to the different effects of alcohol on thin versus normal or fat people affecting their ability to maintain or gain weight. Further metabolic and dietary studies are needed to explain these findings.

Polyenylphosphatidylcholine Attenuates Alcoholic Fatty Liver and Hyperlipemia by Correcting the Ethanol-induced Mitochondrial Dysfunction. K. P. Navder, E. Baraona, and C. S. Lieber. Alcohol Research Center, Bronx VA Medical Center and Mount Sinai School of Medicine, New York, NY.

Dietary supplementation of polyenylphosphatidylcholine (PPC) (a soybean-extracted lecithin, 40–52% of which is dilinoleoylphosphatidylcholine) prevents the development of cirrhosis in alcohol-fed baboons. However, it is unknown whether PPC also affects earlier changes induced by alcohol consumption, such as the fatty liver. To assess this, 28 male littermates were pair-fed 36% of energy either as ethanol or as additional carbohydrate for 21 days. In each group, half were given PPC (3 mg/cal), whereas the other half received equivalent amounts of linoleate and choline. They consumed the same amount of alcohol (15.4 ± 0.5 g/kg/day). In the alcohol-fed rats, PPC decreased ($p < 0.01$) the hepatomegaly (43%), the hepatic accumulation of triglycerides (50%) and cholesterol esters (46%), and the hypertriglyceridemia (50%), whereas it did not affect these values in the pair-fed controls. PPC decreased the ethanol-induced increase in low-density lipoprotein (LDL)-cholesterol without affecting the rise in high-density lipoprotein (HDL)-cholesterol, thereby decreasing further the atherogenic LDL/HDL ratio. PPC did not interfere with fat absorption nor alter plasma free fatty acids, suggesting that PPC acts by decreasing the derangements of fatty acid metabolism produced by ethanol in the liver. We then determined the capacity of liver mitochondria to oxidize fatty acids. As expected, ethanol feeding decreased the capacity of the hepatic mitochondria to oxidize palmitoylcarnitine, with a reduction in respiratory control (47%; $p < 0.01$) and ADP:O ratio (17%; $p < 0.01$). This was associated with decreases in the oxidation of glutamate and succinate, substrates for sites I and II of the electron transport chain, and a 28% diminution in the activity of cytochrome oxidase, a membrane-bound enzyme ($p < 0.01$). PPC abolished the changes produced by ethanol, whereas it had no effect in the controls. The ethanol-induced increases in serum glutamate dehydrogenase and aminotransferases were also corrected by the administration of PPC.

In conclusion, the prevention of alcoholic fatty liver and hyperlipemia by PPC in the rat is caused, at least in part, by its capacity to correct ethanol-induced impairments of mitochondrial fatty acid oxidation in the liver. Such a mechanism may also contribute to the observed prevention of liver injury, including cirrhosis, in alcohol-fed baboons.

Prevention of Hepatic Stellate Cell Activation by Dilinoleoyl Phosphatidylcholine. J. Poniachik, E. Baraona, and C. S. Lieber. Alcohol Research Center, Bronx VA Medical Center and Mount Sinai School of Medicine, New York, NY.

Polyenylphosphatidylcholine (PPC) protects against the development of fibrosis and cirrhosis in alcohol-fed baboons. The mechanism for this protective effect is unknown. Hepatic stellate cells (HSC) are transformed or "activated" to myofibroblast-like cells, which are engaged in the development of fibrosis. The *in vivo* protection by PPC is associated with a 2-fold reduction in the number of transformed cells, suggesting decreased HSC activation. The two major components of PPC are dilinoleoyl phosphatidylcholine (DLPC) (approximately 50%) and palmitoyl-linoleoyl phosphatidylcholine (PLPC) (approximately 24%). To study whether any of these major components of PPC affects activation directly, we assessed expression of α-smooth muscle actin (α-SMA) and collagen synthesis (two recognized manifestations of activation) in primary HSC cultures. HSC were isolated from normal rat livers by density gradient centrifugation after digestion with pronase and collagenase, and then seeded on uncoated plastic dishes and cultured for 8 to 12 days in four different media: a) Dulbecco's modified Eagle's medium with 20% bovine serum; b) the same medium with 17 mM ethanol, used as solvent for the phospholipids; c) medium containing 10 µM DLPC plus 17 mM ethanol; and d) medium containing 10 µM PLPC, also with 17 mM ethanol. After the HSC reached confluence, their expression of α-SMA was quantified by densitometry on Western blots, and their capacity to synthesize collagen was assessed by the 24-hour incorporation of $[^3H]$proline into collagen as well as by the percentage of newly secreted proteins as collagen. Although the number of cells was unaffected by the treatments, DLPC (but not PLPC) decreased α-SMA expression, as judged by a 3- to 4-fold reduction in densitometry units, compared with the other groups ($p < 0.05$). DLPC also decreased the incorporation of $[^3H]$proline into collagen by 40%, compared with the effect of the vehicle alone or with PLPC ($p < 0.05$). The same trend was found for the percentage of proteins secreted as collagen.

In conclusion, DLPC decreased the *in vitro* activation of HSC, as judged by their expression of α-SMA and their capacity to synthesize collagen, whereas PLPC failed to show any effect. The reduction in the activation of HSC by DLPC may contribute to the prevention of fibrosis after administration of PPC *in vivo*.

Plasma Chlorzoxazone Metabolite Correlates with Hepatic Cytochrome P4502E1 in Humans. A. S. Rosman, V. M. Mishin, P. Basu, I. Kessova, C. M. Omata, and C. S. Lieber. Alcohol Research Center, Bronx VA Medical Center and Mount Sinai School of Medicine, New York, NY.

Previous *in vitro* studies have demonstrated that hepatic cytochrome P4502E1 metabolizes chlorzoxazone (CZX, a commonly used muscle relaxant) to 6-hydroxychlorzoxazone (6-OH-CZX). We wished to determine whether measurement of the plasma 6-OH-CZX/CZX ratio after a CZX challenge could serve as a noninvasive marker of hepatic P4502E1 content in patients undergoing liver biopsy. Three subject groups consisting of recently drinking alcoholics (last drink within 24 hours, $n = 6$), abstinent alcoholics (no drink during the preceding 14 days, $n = 5$), and nonalcoholic subjects with liver disease ($n = 5$) were recruited. A liver biopsy was performed for diagnostic purposes and excess tissue was used for immunochemical determination of hepatic P4502E1 content. Within an hour of the liver biopsy, 750 mg of CZX was administered orally and serial plasma samples were collected for 6 hours. The CZX test was also repeated in recently drinking alcoholic subjects after 8 days of observed abstinence. Recently drinking alcoholic subjects had a higher area under the curve for plasma 6-OH-CZX (1354 ± 258 $\mu g \cdot min \cdot L^{-1}$) as compared with abstinent alcoholic patients (296 ± 80 $\mu g \cdot min \cdot L^{-1}$, $p < 0.005$) and with subjects with nonalcoholic liver disease (428 ± 61 $\mu g \cdot min \cdot L^{-1}$, $p < 0.005$). The use of the plasma 6-OH-CZX/CZX ratio at 90, 120, and 180 minutes discriminated between recently drinking alcoholic and nondrinking subjects (abstinent alcoholics as well as nonalcoholic patients with liver disease). Hepatic P4502E1 content significantly correlated with the area under the curve of 6-OH-CZX levels ($r = 0.65$, $p < 0.005$), the maximal 6-OH-CZX concentration ($r = 0.76$, $p = 0.001$), and CZX clearance ($r = 0.79$, $p < 0.001$). In the recently drinking group, the area under the curve for plasma 6-OH-CZX significantly decreased after an 8-day abstinence period (934 ± 175 $\mu g \cdot min \cdot L^{-1}$, $p < 0.05$).

In conclusion, measurement of plasma 6-OH-CZX after administration of a CZX challenge can serve as a noninvasive marker of hepatic P4502E1 activity. This test could be of clinical utility in averting adverse drug reactions secondary to the 2E1 induction, particularly in heavy drinkers.

Ethanol Inhibits Hepatic Microsomal Acetaldehyde Oxidation. Y. S. Wu, K. S. Salmela, and C. S. Lieber. Alcohol Research Center, Bronx VA Medical Center and Mount Sinai School of Medicine, New York, NY.

The microsomal ethanol-oxidizing system (MEOS), inducible by ethanol and acetone, oxidizes ethanol to acetaldehyde (Ach), which causes however, reported that rat liver microsomes also oxidize Ach, thereby challenging the validity of the assessment of MEOS activity by measuring Ach production, and suggesting that MEOS activity may not result in the accumulation of Ach, but rather of its nontoxic metabolic, acetate. To address these issues, we compared the rates of ethanol and Ach oxidation. Male Sprague-Dawley rats (200–250 g) were given acetone (5 mL/kg as a 33% solution i.g. for 3 days) and killed 18 hours after the last treatment. MEOS activity was determined by incubating 0.5 mg microsomal protein with 50 mM ethanol, 1 mM NADPH, 0.1 mM azide, and 0.1 mM desferrioxamine in a final volume of 1 mL for 15 minutes. Ach was measured by head-space gas chromatography with the subtraction of blanks lacking NADPH. Under these conditions, 67 ± 1 µM Ach was produced, reflecting an ethanol oxidation rate of at least 8.9 ± 0.1 nmol/min/mg protein (mean ± SEM of four rats). Under the same conditions (but without ethanol), the rate of disappearance of 50, 100, and 300 µM was significantly less: 0.67 ± 0.01, 1.8 ± 0.1, and 3.6 ± 0.3 nmol/min/mg protein, respectively. Oxidation of Ach to acetate was assessed by incubating microsome with 300 µM $[^{14}C]$Ach, with and without 50 mM ethanol. To eliminate the Ach remaining after the incubation, the samples were centrifuged, the pellet discarded, and the supernatant evaporated to dryness under nitrogen at 60°C; the residue was dissolved in KP buffer, and the radioactivity detected by liquid scintillation. The results were corrected for the dilution of the radiolabeled Ach by the unlabeled Ach produced from ethanol. The rate of $[^{14}C]$Ach oxidation to $[^{14}C]$acetate was 3.8 ± 0.5 nmol/min/mg protein, equivalent to that of Ach disappearance. In the presence of ethanol, however, Ach oxidation decreased to 0.51 ± 0.5 nmol/min/mg protein, which is only 5.7 ± 0.6% of the rate of ethanol oxidation. At Ach levels actually observed in microsomes during ethanol oxidation, an even lower percentage would be expected.

In conclusion, liver microsomes oxidized Ach, but this is inhibited by ethanol. Accordingly, MEOS activity can be accurately assessed by measuring the Ach formed. Furthermore, because Ach production vastly exceeds its oxidation, the net result of MEOS activity is microsomal accumulation of Ach in toxic amounts.

Increased Hepatocellular Fatty Acid Uptake in Fatty Livers Caused by Ethanol Abuse but not Those Caused by Obesity. S.-Z. Zhou, C.-L. Kiang, M. Bradbury, M. Blaise, E. Albanis, M. I. Fiel, D. Stump, and P. Berk. Division of Liver Disease, Departments of Medicine, Biochemistry, and Pathology, Mount Sinai School of Medicine, New York, NY.

Fatty liver (FL) occurs in several clinical settings, of which obesity, diabetes, and ethanol (EtOH) abuse are the most common. To investigate whether increased cellular uptake of long-chain free fatty acids (FFA) contributes to hepatic triglyceride (TG) accumulation and FL pathogenesis in these settings, we studied $[^3H]$oleate uptake kinetics, using a standard rapid filtration assay, in hepatocytes isolated by collagenase perfusion from male rats with several animal models of FL, including: (1) Osborne-Mendel (OM) rats with dietary obesity induced by 8 to 10 weeks of feeding a high-fat diet (55% of calories from lard); (2) homozygous Zucker fatty rats (*fa/fa*) with autosomal recessive obesity caused by a leptin receptor mutation; (3) Zucker Diabetic Fatty rats (*ZDF*) with obesity and noninsulin-dependent diabetes mellitus (NIDDM); and (4) Wistar rats fed the standard, EtOH-containing Lieber-DeCarli diet. Appropriate controls for each group were also studied. FFA uptake data were fitted to the Michaelis-Menten equation using the SAAM program.

Results: In each experimental group, the presence of FL was indicated by increased liver weight, and histological steatosis (hematoxylin and eosin), confirmed with Oil Red O staining and/or increased hepatic TG content. As shown in the table below, there was appreciable variability in the V_{max} for $[^3H]$oleate uptake in the various control groups, reflecting differences in strain, age, and diet. However, compared with the corresponding controls, V_{max} (mean ± SE) was unaltered in the dietary (OM) and genetic (*fa/fa*) models of obesity, and in the diabetic (*ZDF*) animals, although, as reported (Berk et al., 1997; 272:8830), FFA uptake by adipocytes was up-regulated in each instance. By contrast, the V_{max} for $[^3H]$oleate uptake was increased significantly in hepatocytes from EtOH-fed animals compared with control rats receiving a similar but EtOH-free liquid control diet on either a pair fed (PF) or *ad libitum* (*al*) basis. Livers in the PF controls were not fatty.

TABLE

V_{max}-Hepatocellular $[^3H]$Oleate Uptake (pmol/sec/50,000 cells)

Experimental group/diet	V_{max}	Control group/diet	V_{max}	p
OM/high-fat chow	1.9 ± 0.5	OM/normal (nl) chow	1.8 ± 0.3	NS
fa/fa/nl chow	2.6 ± 0.2	Wistar (+/+)/nl chow	2.7 ± 0.6	NS
ZDF/nl chow	2.6 ± 0.4	Wistar (+/+)/nl chow	2.7 ± 0.6	NS
Wistar/EtOH-fed	1.3 ± 0.1	Wistar/EtOH-free (PF)	0.7 ± 0.1	<0.005
		Wistar/EtOH-free (*al*)	0.5 ± 0.1	<0.001

The increased FFA uptake observed in EtOH-induced FL is consistent with the report that HepG2 cells cultured in EtOH exhibit a dose-dependent increase in FFA uptake and TG accumulation, mediated by up-regulation of a specific FFA transporter (Zhou S-L et al., Hepatology 1996;24:128A).

Conclusions: The mechanism of FFA and TG accumulation in FL caused by EtOH differs from that in obesity and NIDDM. Mobilization of peripheral lipid and its subsequent transfer to the liver may play a more important role in the former situation than previously recognized.

Cardiology

Evaluation of Mask Gas Collection for Open Circuit Spirometry in Patients with Heart Failure. D. A. Baran, E. T. Rosenwinkel, J. Whelen, J. Lisker, and R. L. Goldsmith. Division of Cardiology, Mount Sinai School of Medicine, New York, NY.

The measurement of oxygen uptake ($V\mathrm{O}_2$) is important in the evaluation and management of patients with congestive heart failure (CHF). However, the standard mouthpiece-noseclip (MN) device limits oral communication and is uncomfortable for many patients. The Hans-Rudolph facemask (FM) is an alternative device which may alleviate these shortcomings and has produced results similar to MN in healthy subjects. However, the use of FM in patients with CHF has not been validated. Therefore, we compared MN and FM in 12 CHF patients (M = 11, F = 1, age = 52 ± 12 years, peak $V\mathrm{O}_2$ = 17.9 ± 6.1 mL/kg/min). Patients performed a maximal treadmill test with each device in a random order. The tests were performed within 2 weeks of each other. We analyzed $V\mathrm{O}_2$, CO_2 production ($V\mathrm{CO}_2$), ventilation (VE), respiratory exchange ratio (RER), heart rate (HR), and exercise time at the ventilatory breakpoint (VB) and peak exercise. Mean peak RER = 1.11 on both tests (p = 0.86). Shown are mean ± SEM, FM-MN (Δ), and 95% confidence intervals (CI).

Peak	$V\mathrm{O}_2$ (mL/min)	$V\mathrm{CO}_2$ (mL/min)	VE (L/min)	HR (bpm)	Time (s)
FM	1691 ± 240	1864 ± 271	73.6 ± 6.4	151 ± 8	705 ± 69
MN	1652 ± 774	1853 ± 270	66.5 ± 6.5	145 ± 9	646 ± 88
Δ(CI)	39	11	7.1	6	59
	(−56, 135)	(−80, 101)	(1.5, 12.7)*	(−1, 12)	(−7, 125)

VB	$V\mathrm{O}_2$ (mL/min)	$V\mathrm{CO}_2$ (mL/min)	VE (L/min)	HR (bpm)	Time (s)
FM	1209 ± 158	1037 ± 143	36.1 ± 3.2	119 ± 5.7	408 ± 48
MN	1145 ± 44	1008 ± 141	33.1 ± 3.2	115 ± 8.2	373 ± 66
Δ(CI)	64	29	3.1	4	35
	(−94, 223)	(−142, 200)	(−2.9, 9.0)	(−7, 15)	(−37, 107)

* $p < 0.05$.

The confidence intervals demonstrate no clinical difference between FM and MN but show VE, HR, and time at peak exercise to be greater with FM. This positive trend may be related to the greater comfort associated with the FM device. These results indicate that the FM device can be used to accurately measure gas exchange during exercise in patients with CHF.

Left Ventricular Smoke: A Transthoracic Echocardiographic Study. L. Baruch, P. O'Dwyer, P. Patacsil, S. Chockalingam, E. Stern, C. Eng, and M. T. Javed. Division of Cardiology, Bronx VA Medical Center; and Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY.

Spontaneous echo contrast (SEC), smoke is usually seen during transesophageal echocardiography in the left atrium of patients with atrial fibrillation (severe atrial dysfunction) and is associated with thromboembolic events. The incidence and correlates of left ventricular (LV) smoke are unknown. Thromboembolic events are common in patients with congestive heart failure with an annual incidence of 2 to 3%. Transthoracic echocardiography using 2- to 3.5-MHz transducers rarely permits visualization of LV smoke. Higher frequency transducers may permit visualization of LV smoke because the scatter of red blood cells is proportional to the 4th power of the frequency with which they are imaged. We evaluated the ability of a high-frequency 5-MHz pediatric small footprint transducer to detect LV smoke in 710 consecutive patients who underwent transthoracic echocardiography. We compared the 58 patients with smoke (SEC+) with the 652 patients without smoke (SEC−) with respect to LV systolic function as assessed by ejection fraction (EF), LV diastolic function as assessed by transmitral E/A ratio and deceleration time (ms) (DT), pulmonary vein systolic/diastolic ratio (S/D), LV diastolic dimensions (cm) (LVID), and the incidence of thrombi. Fifty-eight patients (8%) had LV smoke.

	Age	EF (%)	LVID (cm)	DT (ms)	Thrombi (%)
SEC+	63.6	37.8	5.6	178.3	3.4
SEC−	61.0	55.9	5.0	217.3	0
p	<0.001	<0.001	0.003	0.004	0.2

Results: Patients with smoke have greater systolic and diastolic dysfunction as manifested by a lower ejection fraction, larger internal diameter, greater E/A and lower S/D ratios than those without smoke. No difference in the incidence of thrombi was noted between the two groups.

Conclusions: The clinical significance of LV smoke, its association with thromboembolic events (as in atrial fibrillation), and the potential benefit of anticoagulants in patients with cardiomyopathy and LV smoke needs to be determined.

Cardioversion of Atrial Flutter: Thromboembolic Potential, ACCP, and TEE, Consensus or Confusion? L. Baruch, S. Vaughn, and A. Klein. Division of Cardiology, Bronx VA Medical Center, Bronx, NY; Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY; and Cleveland Clinic, Cleveland, OH.

The thromboembolic potential of atrial flutter after cardioversion has been assumed to be extremely low. Therefore, the American College of Chest Physicians (ACCP) does not recommend routine pericardioversion anticoagulation of this arrhythmia as it does for atrial fibrillation. However, thromboembolic events and left atrial dysfunction after cardioversion of atrial flutter have been reported. ACUTE is a prospective multicenter study evaluating the utility of transesophageal echocardiography (TEE) in patients undergoing cardioversion of atrial fibrillation. We surveyed 36 of the ACUTE investigators about their experiences and practices when cardioverting atrial flutter concerning the following: 1) performance of TEE before cardioversion (TEE PRE); 2) anticoagulation before and after cardioversion (AC); 3) the need for a change in the current ACCP recommendations (ACCPΔ); 4) identification of precardioversion left atrial or appendageal thrombi; and 5) embolic event after cardioversion. The survey was completed by 32 of 36 investigators.

TEE PRE	28.1%
Anticoagulate	37.5%
ACCPΔ	59.3%

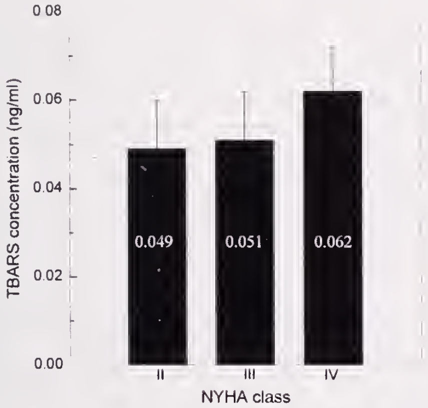
Fifteen investigators (47%) reported a minimum of one thromboembolic event or a left atrial or appendageal thrombus in patients undergoing cardioversion of atrial flutter.

Conclusions: Confusion exists regarding cardioversion of atrial flutter, thromboembolic potential, the role of TEE, and recommendations for anticoagulant therapy. This is likely a result of thrombi or embolic events documented even in patients undergoing cardioversion of atrial flutter. A registry and prospective trial documenting the incidence of thromboembolic events and the potential utility of anticoagulant therapy in patients undergoing cardioversion of atrial flutter are needed to clarify these issues and to achieve a true consensus.

Free Radical Activity Increases with New York Heart Association Class in Congestive Heart Failure. R. H. Charney, D. K. Levy, J. Kalman, C. Buchholz, O. N. Ocampo, C. Eng, and M. L. Kukin. Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY; and Bronx VA Medical Center, NY.

Oxygen free radicals have been implicated in the genesis and progression of congestive heart failure (CHF). It has been demonstrated that thiobarbituric acid-reactive substances (TBARS), which are markers of lipid perioxidation, are elevated in patients with CHF, compared with controls.

In this study, we correlated TBARS with New York Heart Association (NYHA) functional class. TBARS were measured by spectrophotometry in the plasma of 54 patients with CHF. The mean age of the patients was 61 years (range, 29–87) and the mean baseline ejection fraction was 19% (range, 5–38). Ten patients were NYHA class II, 32 were class III and 12 were class IV. There were 46 males and 8 females, of whom 21 had an ischemic etiology, 28 had idiopathic CHF, and 4 had prior valvular heart disease. As shown in the graph (mean TBARS concentration ± SD), there was a progressive increase in TBARS with NYHA class; $p = 0.0211$ by ANOVA. Thus, patients with CHF have increased oxygen free radical production and/or decreased reducing ability. TBARS directly correlated with worsening functional class. This may have therapeutic and prognostic implications in CHF.



Lipoprotein Lp(a) in Human Coronary Atheroma Correlates with Macrophage Infiltration and Clinical Syndromes of Severe Unstable Angina. G. Dangas, R. Mehran, S. K. Sharma, P. Meraj, P. C. Harpel, J. A. Ambrose, and J. T. Fallon. Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY.

Background: Lipoprotein Lp(a) is considered atherogenic, and also thrombogenic because of a structural homology to plasminogen. We investigated the association of Lp(a) with macrophages in coronary atheroma obtained from living patients who underwent directional atherectomy.

Methods: We studied tissue specimens removed from 72 patients. Paraffin sections from routinely processed specimens were stained with an immunopurified polyclonal anti-human apoprotein(a) antibody, with a monoclonal antibody (A6) directed toward kringle 4 of Apo(a), with an anti-human panmacrophage antibody (anti-CD68; KP-1), and with an anti-human α-actin antibody, using a biotin-strepavidin-peroxidase technique. Total plaque area and fractional areas of Lp(a), KP-1, and α-actin positive staining were determined by planimetry. Subsequently, the percent KP-1/Lp(a) and the percent α-actin/Lp(a) overlap was determined.

Results: All specimens had localized Lp(a) staining; 82% of specimens stained positively for KP-1. The fractional area of Lp(a) positive staining was 58 ± 4%, of KP-1 25 ± 3%, and of α-actin 41 ± 3% (mean ± SE). There was remarkable colocalization of KP-1 staining with Lp(a) in all specimens. Quantitatively, 89 ± 2% of the KP-1 staining occurred on Lp(a) positive areas ($p < 0.001$), whereas only 31 ± 4% of α-actin colocalized with Lp(a) ($p < 0.001$ for the negative association). The Lp(a) positive area was in 47 ± 25% of patients with stable angina versus 71 ± 18% with class III unstable angina ($p < 0.05$), whereas the KP-1 positive area was in 18 ± 22% of patients with stable angina whereas 31 ± 24% of patients with Braunwald class III unstable angina ($p = 0.05$).

Conclusions: An association between Lp(a) and macrophages in the atheromatous plaque is suggested. Because macrophages have been associated with acute syndromes, the proximity of Lp(a) to the rupture site may contribute to increased local thrombogenicity. Vascular smooth muscle cells, as assessed by α-actin staining, were predominantly localized in the Lp(a) negative areas. The important role of Lp(a) in atherosclerosis is further supported by its localization to a greater extent in patients with unstable versus stable syndromes.

Effect of Long-Term Eprosartan vs Enalapril Antihypertensive Therapy on Left Ventricular Mass and Coronary Physiology. J. Diamond, J. Gharavi, J. Machac, M. Henzlova, and R. Phillips. Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY. The effects of antihypertensive treatment with angiotensin receptor blockers versus angiotensin-converting inhibitors on left ventricular (LV) mass and coronary flow reserve were studied. Seventeen hypertensive patients (9 M, 8 F; untreated diastolic blood pressure (BP) ≥100 mm Hg) were randomly assigned in a double-blind fashion to eprosartan or enalapril. The extent of BP reduction, LV mass regression, and change in coronary flow reserve (CFR) were measured after a 2-week placebo period (base), after a 3- to 18-week titration (titr) period, and after another 2 to 3 months treatment (Rx). LV mass was measured by echo. CFR was quantified by split-dose thallium-201 perfusion imaging. The ratio of anterior, septal, inferior, and lateral segment counts during coronary vasodilatation by i.v. adenosine (170 g/kg/min), divided by baseline counts, defined CFR.

	All subjects	Eprosartan	Enalapril
Base office BP mm Hg	154 ± 12*/ 98 ± 10	154 ± 11/ 97 ± 11	153 ± 14/ 99 ± 10
Base-titr office BP	−27 ± 14*/ −16 ± 15*	−26 ± 16*/ −13 ± 14*	−29 ± 13*/ −20 ± 16*
Base-Rx office BP	−17 ± 18*/ −9 ± 14*	−16 ± 18/ −9 ± 17*	−18 ± 21/ −8 ± 16
Base LV mass[a]	115 ± 38	111 ± 37	84 ± 28
Base-titr LV mass	−23 ± 39*	−7 ± 26	−40 ± 42*
Base-Rx LV mass	−17 ± 26*	−10 ± 31	−26 ± 16*
Base CFR[b]	1.47 ± 0.31	1.53 ± 0.34	1.42 ± 0.30
End Rx CFR	1.46 ± 0.32	1.61 ± 0.30**	1.29 ± 0.27**
Base-titr CFR	+0.02 ± 0.39	+0.01 ± 0.29	+0.02 ± 0.48
Base-Rx CFR	−0.04 ± 0.47	+0.08 ± 0.19	−0.17 ± 0.49

[a] LV mass (indexed to height, g/m by PENN convention) = $1.04\ [(IVS + LVID + PWT)^3 - (LVID)^3] - 13.6$.

[b] Inferior segment (representative of other segments) $^{*}p \leqslant 0.05$ for change over time; $^{**}p \leqslant 0.05$ eprosartan vs enalapril.

Thus, after 6 months treatment of arterial hypertension with eprosartan versus enalapril, there was an equivalent reduction in BP. LV mass regression reached statistical significance in the enalapril group but not in the eprosartan group. In contrast, there was a trend toward increase in CFR in the eprosartan group, which was not observed in the enalapril group. Further investigation of the effects of angiotensin receptor blockers on CFR and LV mass regression appear warranted.

Nighttime Ambulatory Systolic Blood Pressure is an Independent Predictor of Diastolic Function. J. A. Diamond, J. S. Jhang, and R. A. Phillips. Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY.

Abnormal left ventricular filling is an early marker of the effects of essential hypertension on the heart. We studied 115 hypertensive paients (76 M, 39 W; mean age, 44 ± 11 years) referred for clinic blood pressure (CBP) ≥140/90 mm Hg to determine the relationship between clinic, 24-hour ambulatory BP (ABP), daytime ABP, or nighttime ABP and Doppler echocardiographically measured ratio of late to early diastolic flow velocity (A/E ratio). The patients had no other cardiovascular disease and had not received antihypertensive medication for at least 2 weeks before entering the study. ABP recordings were obtained with a Spacelabs monitor. Doppler echocardiograms and 2D and M-mode echocardiograms were obtained using an ATL Ultramark 6 scanner.

The mean blood pressure was 149 ± 26/93 ± 18 mm Hg for clinic BP, 146 ± 20/92 ± 15 mm Hg for 24-h ABP, 148 ± 19/94 ± 15 for daytime ABP, and 135 ± 24/81 ± 19 for nighttime ABP. The A/E ratio significantly correlated with all measures of systolic and diastolic BPs. The A/E ratio also correlated with age ($n = 0.59$, $p < 0.0001$), body mass index ($r = 0.24$, $p < 0.01$), heart rate ($n = 0.50$, $p < 0.0001$), and left ventricular mass indexed for height ($r = 0.30$, $p < 0.0009$). Abnormal diastolic function (A/E $\geqslant 1$) was found in 38/115 (33%) of subjects. Stepwise, forward multivariate analysis demonstrated that the A/E ratio was best predicted by age and heart rate. After accounting for these variables, mean nighttime ABP remained, to a lesser extent, a significant and independent predictor of LV filling ($p < 0.02$).

Both clinic and ambulatory blood pressures correlate with the Doppler determined A/E ratio. Although A/E ratio correlates best with age and heart rate at time of echocardiographic measurement, there is still an independent correlation with mean nighttime systolic ABP. This suggests that nighttime blood pressure load on the heart affects diastolic function. The extent to which this influences long-term morbidity remains to be determined.

Effect of Long-Term Nifedipine GITS versus Verapamil SR on Clinic, Ambulatory, and Exercise Blood Pressure in Patient with Moderate to Severe Hypertension. J. Diamond, L. Krakoff, and R. Phillips. Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY.

We studied the efficacy of Nifedipine GITS versus Verapamil SR in controlling ambulatory and peak exercise blood pressure (BP) in untreated patients with ≥stage II hypertension. Thirty hypertensive patients (19 men, 11 women) were randomly assigned to maximally required doses of either Nifedipine GITS or Verapamil SR to lower diastolic BP to ≤95 mm Hg. The extent of clinic BP, 24-hour ambulatory, daytime, nighttime, and peak exercise BP and HR changes were measured at baseline and after 6 months of treatment. Subjects underwent ambulatory BP monitoring and symptom-limited bicycle exercise testing after a 2-week placebo phase, and after 6 months of treatment. Ambulatory monitoring was performed using the SpaceLabs 90202 or 90207 model. Groups were compared using a student's *t*-test. Baseline clinic, ambulatory, and exercise BPs were similar in both groups.

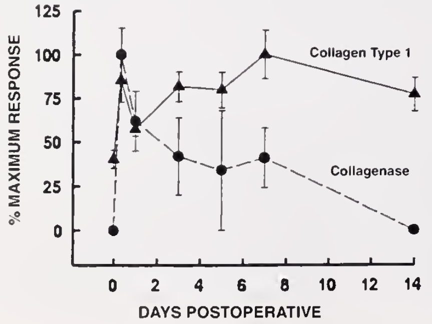
	Verapamil SR	Nifedipine GITS
Blood pressure (mm Hg)		
Δ clinic BP	−25 ± 6/−17 ± 3	−21 ± 4/−15 ± 2
Δ 24-hour BP	−16 ± 3/−13 ± 2	−21 ± 2/−15 ± 2
Δ day BP	−18 ± 3/−14 ± 2	−22 ± 2/−16 ± 2
Δ night BP	−11 ± 3/−10 ± 2	−16 ± 3/−10 ± 2
Δ exercise BP†	−15 ± 5/−15 ± 6	−33 ± 6/−14 ± 6*
Heart rate (bpm)		
Δ 24 hour HR	−7 ± 6	+1 ± 10*
Δ day HR	−8 ± 6	+1 ± 12*
Δ night HR	−3 ± 8	+1 ± 8
Δ exercise HR†	−17 ± 12	+0.3 ± 11**

* $p \leqslant 0.03$; **$p \leqslant 0.0004$ for nifedipine GITS vs verapamil SR.
† There was no significant difference in double product, total exercise time, or maximal watts achieved for the patient groups.

During 6 months, there were similar reductions in clinic BP and ambulatory BP. Both drugs effectively controlled BP in patients with ≥stage II hypertension. Nifedipine GITS was more effective in lowering peak exercise systolic BP. However, Verapamil SR produced significantly lower heart rates at rest and with exercise. The long-term effects of these changes on cardiovascular morbidity and mortality need to be evaluated further.

Early Gene Expression for Collagenase and Collagens I and III in Volume-Overload Cardiac Remodeling. S. Dolgilevich, F. Siri, S. Atlas, and C. Eng. Bronx VA Medical Center and Mount Sinai School of Medicine, New York, NY.

Chronic cardiac volume overload induces marked ventricular dilation and wall remodeling, but the mechanisms involved in this transition are not well understood. Northern blot analysis with cDNA probes for collagenase (CGSE) and collagen types I (Coll I) and III (Coll III) was used. Total RNA was extracted from unoperated (N, $n = 7$) and sham-operated (SH, $n = 5$) controls. CGSE was undetectable in SH but significantly expressed in AVF ($p < 0.01$ vs SH). Coll I and Coll III were both significantly higher in AVF vs N ($p < 0.05$ in both cases). CGSE expression gradually declined thereafter, whereas Coll I peaked at 248% of N at 7 days ($p < 0.01$) and remained significantly elevated at 14 days ($p < 0.05$). Thus, important genes affecting the extracellular matrix are activated early in cardiac volume-overload remodeling.



High Resolution Magnetic Resonance Imaging of Atherosclerotic Lesions in Apolipoprotein E-deficient Mice. Z. A. Fayad,[1,2] M. Shinnar,[1] V. Fuster,[1] S. Wehrli,[3] H. M. Dansky,[4] M. Poon,[1] J. J. Badimon,[1] E. A. Fisher,[1] and J. T. Fallon.[1,5] [1]Cardiovascular Institute, and Departments of [2]Radiology and [5]Pathology, Mount Sinai School of Medicine, New York, NY; [3]Division of Biochemical Development and Molecular Diseases, Children's Hospital of Philadelphia, Philadelphia, PA; and [4]Laboratory of Biochemical Genetics and Metabolism, The Rockefeller University, New York, NY.

Background: The pathogenesis of complex cardiovascular and pulmonary diseases are currently being investigated in genetically engineered small animals. Methods to follow the time course of the developing pathology and/or the response to therapy *in vivo* have been limited.

Methods: To address this problem, we developed a noninvasive, magnetic resonance (MR) microscopy technique to study atherosclerotic lesions *in vivo* in the apolipoprotein E-deficient (apoE$^-$) mice.

Results: The lumen and the vessel wall of the abdominal aorta and common iliac arteries in apoE$^-$ and control mice, receiving chow diet, were identified on all images. Atherosclerotic lesions, when present, were detected and characterized using high-resolution MR and the findings were correlated closely with histopathology.

Conclusion: The combination of MR microscopy and genetically engineered animals is a powerful tool to investigate the development of atherosclerotic lesions in the intact animal serially and noninvasively and should greatly accelerate knowledge of atherogenesis.

Apoprotein B Can Be Reversibly Ubiquitinated During Translation and Translocation: A New Paradigm for Proteasomal Degradation of a Secretory Protein. E. A. Fisher,[1] D. M. Mitchell,[1] M. Zhou,[2] H. Wang,[1] J. Aitchison,[3] and H. N. Ginsberg.[2] [1]Mount Sinai School of Medicine, New York, NY; [2]Columbia University, New York, NY; and [3]University of Alberta, Canada.

Liver-derived apoprotein B (apoB) is the major protein of atherosclerosis-causing lipoproteins. In HepG2 cells, a model of the human liver, 60% of newly synthesized apoB is degraded intracellularly within 20 min. We showed previously that apoB degradation is mediated by the ubiquitin (Ub)-proteasome pathway and involves cytosolic Hsp70. In the present studies, cells were pretreated with the proteasome inhibitor lactacystin and then labeled (15 min) with ^{35}S-Met. One hour later, oleic acid (OA) was added to stimulate lipid synthesis and lipoprotein assembly/secretion, and labeled apoB in cell lysate and medium was measured during 150 min. Although lipid synthesis was stimulated well after apoB translation, labeled apoB was secreted quantitatively as a lipoprotein. As shown by cross-linking, apoB interacted similarly with the translocon protein sec61 during apoB translation and after 20 min or 1 hour chase. Cell fractionation showed that during the chase, apoB was microsomal and did not accumulate in the cytosol. Also, when OA was added, apoB had already been conjugated to Ub, as detected by immunoprecipitation with anti-Ub and anti-apoB antibodies; secreted apoB, however, was not ubiquitinated. Finally, partially translated Ub-apoB species were observed when a puromycin-induced translational block was released in the presence of ^{35}S-Met. These data indicate that apoB can be targeted to the Ub-proteasome pathway as early as translation and can remain associated with the translocon. If the lipid supply is adequate, apoB can be de-ubiquitinated and secreted as a lipoprotein.

Coordinated Expression of the Type 1 Inositol 1,4,5-Triphosphate Receptor and Apoptosis in the Developing Mouse Embryo. D. E. Gutstein, M. Moschella, T. Jayaraman, H. A. Skopicki, and R. Marks. Program in Molecular Cardiology, Columbia University College of Physicians and Surgeons, New York, NY; and Division of Cardiology, Mount Sinai School of Medicine, New York, NY.

The inositol 1,4,5-trisphosphate receptor (IP3R) is a calcium release channel located on the endoplasmic reticulum of most cell types. Calcium signaling mediated by IP3Rs regulates a wide variety of physiological processes. In our studies, we have focused on the role of the IP3R in apoptosis. Our laboratory has previously reported that the IP3R is up-regulated in human heart failure, a condition associated with cardiomyocyte apoptosis. In our series of explanted human hearts, apoptosis was readily detected in 75% of hearts from patients with ischemic cardiomyopathy ($n = 8$) and 17% of those with idiopathic dilated cardiomyopathy ($n = 6$), but not in nonmyopathic hearts ($n = 2$). Our laboratory has also shown that a T-lymphocyte cell line stably transfected with an antisense fragment to the 5′-most portion of the type 1 IP3R cDNA resists apoptosis induced by glucocorticoids, ionizing radiation, αCD3 and αCD95. These data suggest that the IP3R may play a critical role in apoptotic signaling. To study the relationship between the IP3R and apoptosis further, we studied the developmental regulation of type 1 IP3R mRNA expression and apoptosis in the mouse embryonic hindlimb. IP3R1 mRNA was detected in the murine hindlimb interdigital regions at day 14.5 postimplantation and correlated with the appearance of apoptotic nuclei, whereas IP3R1 mRNA was not observed in adjacent tissue which does not undergo apoptosis. Thus, it appears that the IP3R may play a role in apoptosis in the developing mouse hindlimb.

Calcium Channel Blocker Use in Late 1996 by Physicians in a Tertiary Care Medical Center. J. S. Jhang, S. Patel, and R. A. Phillips. Hypertension Section, Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY.

In light of recent studies suggesting increased morbidity and mortality with short-acting calcium channel blockers (CCBs), changes in prescription of long-acting CCBs was assessed in 175 physicians. A cross-sectional survey was conducted in October 1996 at Mount Sinai Medical Center, New York, NY using a questionnaire. Of the physicians, 49.1% reported that they had taken patients off of long-acting CCBs because of safety concerns. However, physicians did not report any change in the percentage of patients receiving CCBs at the present time versus 6 months ago (146 of 171 respondents). Of those physicians reporting a change, a significantly greater number reported a decrease versus an increase (Wilcoxon signed rank test = 107.5, $p = 0.0007$). The three most frequently chosen reasons for taking patients off of long-acting CCBs were: the patient was not tolerating the nonfatal adverse effects of CCBs, the patient was concerned about the safety of CCBs, and studies raised a doubt regarding the safety of CCBs. Despite the concern about the safety of CCBs, physicians reported that they believed that short-acting CCBs should be available for hypertensive crisis and that CCBs should not be removed from the market entirely. This study demonstrates the need for prospective randomized clinical trials to determine the long-term effects of these agents.

Should Low Blood Pressure Preclude Initiation of Beta Blocker Therapy in Congestive Heart Failure. J. Kalman, D. K. Levy, M. M. Mannino, R. S. Freudenberger, C. Buchholz, O. N. Ocampo, and M. L. Kukin. Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY.

Evidence is now accumulating of the benefit of β-blocker (β-B) therapy in refractory heart failure. However, many physicians are understandably reluctant to initiate β-B therapy when the systolic blood pressure (SBP) is below 100 mm Hg. We compared the safety and efficacy of metoprolol according to the initial BP in 57 consecutive patients enrolled in β-B protocols.

All patients were receiving chronic background therapy (digoxin, diuretics, and angiotensin-converting enzyme inhibitors) with a pulmonary capillary wedge pressure (PCW, mm Hg) ≥14. Sixteen patients had SBP ≤ 100 (low BP) and 41 had SBP > 100. Patients were New York Heart Association (NYHA) II–IV, with 70% in NYHA III. Aside from BP-derived variables, baseline parameters were similar in both groups. Baseline (B) and 3-month (LT) variables of SBP, heart rate (HR, bpm), PCW, systemic vascular resistance (SVR, d-s-c), stroke work index (SWI, gm · m/m^2), and ejection fraction (EF) are shown (*$p < 0.05$ vs B within group, #$p < 0.05$ between group B to LT).

	SBP	HR	PCW	SVR	SWI	EF
Low BP: baseline	94	89	26	1211	16.5	10
Low BP: long-term	101*	67*	18*	1055	31.5*	14*
BP > 100: baseline	118	88	28	1330	23.1	13
BP > 100: long-term	112*#	71*	21*	996*	32.4*	18*

Nine patients did not complete the study (5: SBP > 100, 4: Low BP; p = NS). The average decrease in NYHA was one class whereas submaximal and maximal exercise increased in both groups (between groups, p = NS).

In summary, both groups improved with β-B therapy with no between group difference in response other than the surprising increase in SBP in the low BP group. Thus asymptomatic hypotension does not preclude initiation or response to β-B therapy.

Cloning of the Rat SM-20 Promoter and Its Differential Expression in Smooth Muscle Cells and Fibroblasts. K. Menzies, S. Wax, and M. Taubman. Division of Cardiology, Department of Medicine, Mount Sinai School of Medicine, New York, NY.

A major goal in the treatment of atherosclerotic disease is to inhibit smooth muscle cell (SMC) proliferation and migration without affecting other cellular components of the vessel wall. A regulatory element that confers SMC-restricted expression might be useful in gene therapy to specifically target vascular SMC. SM-20 is a novel growth factor-responsive gene recently cloned in our laboratory from rat aortic SMC. SM-20 encodes a 239-amino-acid cytoplasmic protein that is expressed in vascular SMC but not in fibroblasts, endothelial cells, or macrophages. We hypothesized that the regulatory region of the SM-20 gene contains *cis*-acting element(s) conferring smooth muscle-restricted expression. To this end, we isolated four overlapping genomic clones encoding SM-20. Restriction mapping and sequence analysis of these clones demonstrated that the SM-20 gene has five exons and spans 30 kb. The putative transcription start site was identified by comparing cDNA and genomic sequences and was confirmed by RNase protection assay. The start site is located 222 bases upstream of the first in-frame ATG. Sequence analysis showed a TATA-less promoter 94-bp upstream of the transcription start site. These upstream sequences contain the following promoter elements: SP1, AP1, AP2, AP4, cMYB, and GATA-1. The 5′-untranslated region contains a MyoD/E47 site as well as AP2 and AP4 sites. A 4.0-kb promoter region was cloned into the pXP2 luciferase reporter plasmid (pXP2-SM20). In transient transfections of rat aortic SMC, the activity of this chimeric construct (normalized to cotransfected human growth hormone) was 37% of that seen with the CMV promoter-luciferase construct, indicating that the SM-20 promoter is highly active. The normalized activity of pXP2-SM20 in mouse NIH 3T3 fibroblasts was 5% of that seen in SMC. These results suggest that the 4.0-kb promoter fragment contains a smooth muscle-specific element(s). Further delineation of this element should provide new insights into SMC-specific gene regulation.

The Relationship Between QT Interval Dispersion and Parameters Derived from Signal-Averaged Electrocardiography. A. O'Keefe, Jr., V. A. DeBari, and E. E. Federici. Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ and St. Elizabeth's Hospital, Elizabeth, NJ.

We sought to: (a) determine whether filtered QRS duration (fQRS), duration of HFLA signals below 40 μv (LAS), and root-mean-square voltage of the terminal 40 ms of the filtered QRS duration (RMS40) derived from the signal-averaged electrocardiogram (SAECG) correlate with QT dispersion on the standard 12-lead electrocardiogram in patients with coronary artery disease; (b) establish a preliminary cutoff for a value of fQRS and test its ability to predict QT dispersion ≥40 ms. The prospective study recorded both standard 12-lead and SAECGs of 29 consecutive patients with coronary artery disease. Patients were studied at rest free of antiarrhythmic drugs, and without evidence of acute myocardial infarction or unstable angina. Standard 12-lead ECGs were enlarged, QT intervals for each lead measured, and QT dispersion calculated. SAECGs were simultaneously recorded and electronically analyzed using a MAC-15 computerized high-resolution electrocardiograph. Linear regression of fQRS, RMS40, and LAS indicate that the strongest correlation existed with fQRS ($r = 0.73$, $p \leq 0.001$); RMS40 showed a significant inverse correlation ($r = 0.48$, $p < 0.05$) but had little predictive value ($r = 0.23$); LAS correlated poorly ($r = 0.25$, not significant). We tested the ability of the fQRS to predict QT dispersion at the 95%, 98%, and 99% confidence interval (CI) of the observed distribution using 2 × 2 contingency tables as a model of logistic regression. These correlations were strong, with $p = 0.004$ at the 95% CI and $p < 0.001$ at the 97% and 99% CI. QT interval dispersion may provide a simple, inexpensive, non-invasive method of measuring underlying dispersion of recovery of ventricular excitability and be a further marker for predicting susceptibility to ventricular tachyarrhythmias.

Identification of a Dexamethasone-Sensitive mRNA-Destabilizing Region in JE/MCP-1. M. Poon, B. Liu, and M. B. Taubman. Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY.

The monocyte chemotactic factor, JE/MCP-1, plays an important role in recruiting monocytes to atherosclerotic and injured arteries. Previously, we showed that platelet-derived growth factor (PDGF) increases and dexamethasone (dex) decreases JE/MCP-1 mRNA stability in cultured rat aortic smooth muscle (SM) (Poon et al. J Biol Chem 1991; 266:22375–22379). Using RNA mobility shift assays, we also identified two regions in the JE/MCP-1 mRNA involved in mRNA stabilization. To further delineate the role of the regions in regulating JE/MCP-1 mRNA stability in response to PDGF and dex, we performed *in vitro* decay assays and deletion analyses. Labeled *in vitro* transcribed JE/MCP-1 RNA was incubated with S-100 cytoplasmic extracts from untreated SM (C) or SM treated with 20 ng/ml PDGF BB ± 10 nM dex and examined at varying times by polyacrylamide gel electrophoresis. Full-length JE/MCP-1 mRNA decayed with a $t_{1/2}$ of 30 minutes for C, 2 hours for PDGF, and 10 minutes for PDGF+dex. A 224-nucleotide (nt) region from the 5′-end and a 170-nt region from the 3′-end of the JE/MCP-1 mRNA both caused gel shifts with PDGF-treated extracts and remained stable with $t_{1/2}$ similar to the full-length JE/MCP-1. However, only the 224-nt region remained dex-sensitive and decayed rapidly in dex with a $t_{1/2}$ of ≈10 minutes. An 80-nt deletion from the 5′-end of the 224-nt region completely abolished the dex effect. The resultant 144-nt fragment remained responsive to PDGF with $t_{1/2}$ of ≈2 hours. These results suggest that different regions of the JE/MCP-1 mRNA are responsible for the PDGF and dex effects on the mRNA stability. Furthermore, a dex-sensitive region appears to be localized to the first 80-nt of the 5′-end of the mRNA. These studies suggest a novel mechanism by which dex regulates mRNA accumulation and the inflammatory response.

Effect of Lipid-Lowering Therapy on Thrombus Formation in Hypercholesteremic Patients. U. Rauch, J. H. Chesebro, D. Vorchheimer, I. Guzman, K. Harris, P. Harris, D. Sandler, F. Bilyk, S. Jayaraman, R. Harris, J Robbins, J. Fallon, V. Fuster, and J. J. Badimon. Cardiovascular Research Laboratory, Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY.

Plasma lipid level reduction is associated with significant improvement in clinical outcome of patients with coronary artery disease (CAD) despite a small effect on angiographic plaque regression. We investigated whether thrombus formation is modulated by lipid-lowering therapy with Simvastatin. We studied 15 patients (10 patients with and 5 without CAD) with total cholesterol (TCHO) > 220 mg/dL; LDL > 145 mg/dL. Thrombus formation was evaluated in an *ex vitro* perfusion system. Nonanticoagulated blood was perfused directly from the patient's arm into the chamber. Pig tunica media served as thrombogenic substrate and thrombus formation was measured as substrate area covered by thrombus. The perfusion studies (5 min; high shear rate) were performed at baseline and 3 months after treatment with Simvastatin. TCHO and LDL were reduced after treatment (for TCHO from 261.1 ± 55.9 mg/dL to 172.3 ± 20.6 mg/dL and for LDL from 194.3 ± 52.8 mg/dL to 107.5 ± 18.1 mg/dL, $p \leqslant 0.01$, respectively). Thrombus deposition on pig tunica media was determined as shown below.

	All patients	CAD	No CAD
Baseline	10317 ± 879	10482 ± 1151	9988 ± 1454
After treatment	83667 ± 497*	8605 ± 528*	7891 ± 1112*

Data are expressed as area platelet deposition ($\mu m^2/mm$) on pig tunica media. Values are mean ± SEM; $^*p \leqslant 0.05$ vs baseline.

Conclusions: A decrease in plasma lipid levels is associated with a significant reduction of thrombus formation, as measured in an *ex vivo* perfusion chamber. Therefore, the observed beneficial clinical effects of lipid-lowering drugs in patients with CAD may be mediated not only through the hypolipidemic effects but also by reducing the thrombogenicity of the circulating blood.

Threshold Inhibition of Thrombus Formation by PEG-Hirudin, A Longer Acting Thrombin Inhibitor. M. Roque,[1] A. F. Alzueta,[1] A. Ratant,[1] K. Rubsamen,[2] L. Badimon,[1] V. Fuster,[1] J. H. Chesebro,[1] and J. J. Badimon.[1] [1]Cardiovascular Institute, Mount Sinai School of Medicine, New York, NY; and [2]KNOLL AG Ludwigshafen, Germany.

A recent meta-analysis of clinical trials using Hirudin reported a 52% reduction of the acute thrombotic complications during its administration. However, its efficacy is limited by its rapid elimination. PEG-Hirudin, a new and longer lasting antithrombin agent, has the advantage of being active after i.v. or s.c. administration. PEG-Hirudin half-life after s.c. injection is 9.5 hours vs 2 hours for r-Hirudin. We compared the antithrombotic effects of PEG-Hirudin with heparin on reducing acute thrombus formation under flow conditions.

Methods: Thrombus formation in the Badimon perfusion chamber was triggered by using severely injured arterial wall (type III lesion). Blood was perfused for 5 minutes at shear rate conditions typical of mildly stenosed coronary arteries. Thrombus formation was quantitated by 111-Indium-labeled platelets. Results are expressed as platelets × $10^6/cm^2$. ATU/mL and μg/PEG-Hirudin mL, respectively.

Treatment group	*n*	Platelets	anti IIa	Plasma levels
Heparin (100 U/kg)	10	432 ± 42	8 ± 1	0
PEG-Heparin, 0.3 mg/kg	10	424 ± 54	61 ± 4*	2 ± 0.1
PEG-Hirudin, 0.6 mg/kg	15	387 ± 42	108 ± 14*	5 ± 0.3
PEG-Hirudin, 0.7 mg/kg	15	343 ± 53	132 ± 9*	6 ± 0.3
PEG-Hirudin, 0.8 mg/kg	15	59 ± 5*	186 ± 16*	10 ± 0.4
PEG-Hirudin, 1.0 mg/kg	15	54 ± 6*	261 ± 29*	17 ± 1.0

Mean ± SEM; $^*p < 0.05$ vs heparin. PEG, polyethylene glycol; ATU, anti IIa units

Specific thrombin inhibition by PEG-Hirudin significantly reduces platelet-thrombus formation. The inhibitory effects follow a threshold dose response unlike the direct relationship previously seen with heparin. Thus, an adequate and maintained thrombin inhibition may play a significant role in the prevention of thrombosis in these clinical conditions characterized by high shear rate and deep arterial injury (plaque disruption, percutaneous transluminal coronary angioplasty).

Transient Attenuation of Infarctional Q-Waves from Exercise-provoked Ischemia in the Heart Region Opposite to Necrotic Scar: A New Concept Leading to an Enhancement in Diagnostics. N. Solanki, C. Tziros, V. Singh, M. Khalil, P. Knez, and J. A. Madias. Mount Sinai School of Medicine, Elmhurst Hospital Center, New York, NY.

Recent observations from our laboratory (J Electrocardiol 1997;30:97) indicate that patients with myocardial infarction (MI) undergoing exercise/thallium testing (ETT/TI) may display transient loss of Q-waves in their electrocardiogram, when reversible ischemia (IS) of the opposite to the MI myocardial region (OMR) is evident. This phenomenon constitutes an interaction of permanently necrotic myocardium from the prior MI with a transiently ischemic region of the heart, resulting from the exercise testing. The concept underlying this phenomenon is at variance with the concept that only ST-segment displacement can be taken as an index of ischemia. Q-waves in the setting of MI are based on the depolarization of the noninfarcted myocardium opposite the area of necrosis; when such a region is rendered ischemic during ETT/TI, it may become incapable of generating strong depolarization forces pointing away from the scar territory, and thus Q-waves either disappear or they diminish in amplitude. Consequently, we investigated whether patients with the above attributes show modification of the Q-wave amplitude during ETT/TI. To this end, we performed ETT/TI in a consecutive series of 183 patients with a history of MI, Q-waves in their electrocardiograms, and fixed TI defect at rest. The patients were allocated into those with IS in the OMR (+ISOMR), and the ones without IS in such a territory (−ISOMR), based on the TI results. Data from Q-waves in mm at rest (ΣQR), at peak exercise (ΣQP), and percent change between the two measurements (%ΔΣQ) were (mean ± SD):

	n	ΣQR	ΣQP	%ΔΣQ
−ISOMR	107	$21.5 \pm 12.2^{a,c}$	$22.3 \pm 12.5^{e,c}$	7.3 ± 21.9^{b}
+ISOMR	76	$26.8 \pm 14.4^{a,d}$	$17.3 \pm 11.7^{e,d}$	-36.9 ± 17.4^{b}

$^{a,c,e}\ p > 0.05$; $^{b,d}p < 0.0005$.

We conclude that IS in the OMR may lead to temporary partial reduction of the amplitude of infarctional Q-waves during ETT/TI; the postulated explanation is an "electrical inertia" which, during exercise, results transiently in attenuated generation of depolarization forces by the OMR, as compared with the ones produced at rest.

Identification of Extracellular Tissue Factor Released from Human Aortic Smooth Muscle Cells. B. D. Spirn, A. D. Schecter, P. L. A. Giesen, R. E. Gordon, J. T. Fallon, Y. Nemerson, and M. B.

Taubman. Divisions of Cardiology, Thrombosis Research, and Pathology, Mount Sinai School of Medicine, New York, NY.

Tissue factor (TF), the initiator of coagulation, is a transmembrane protein whose biologic function is thought to occur predominantly at the cell surface. Our laboratory has examined the expression of TF protein in human atherosclerotic plaques and in rat and rabbit models of arterial injury. In the plaque, most TF is found within the acellular lipid-rich core and fibrous extracellular matrix (ECM). In the injured arterial media, TF is present in smooth muscle cells (SMC) and in the surrounding ECM. These data suggest that TF may be released from SMC into the ECM. To examine this, TF activity was measured in the medium of cultured human aortic SMC. Cells were grown on 12-well culture plates in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (FBS). Forty-eight hours after feeding, the medium was removed, the plates were washed twice with phosphate-buffered saline and incubated in serum-free medium. At varying times, aliquots of the medium were removed and examined for TF activity. TF activity (Factor Xa [FXa] generation) was determined by chromogenic assay. FXa values were converted to femtomoles of TF assuming a K_{cat} for the TF/FVIIa complex of 300 min^{-1} at 20°C. Immediately after washing (0_t), the amount of TF activity measured was minimal (0.57 ± 0.40 fmol). However, TF activity in the medium rose linearly during 30 min to 3.44 ± 1.31 fmol. TF activity rose more slowly between 30 minutes and 48 hours to a maximum value of 6.72 ± 3.30 fmol. Levels of lactate dehydrogenase measured by chromogenic assay were negligible. This suggests that cell death was not responsible for TF measured in the medium. TF requires the presence of phospholipids to be enzymatically active. To understand the structure with which the active TF is associated, the medium was subjected to differential centrifugation. 12% of the TF activity was pelleted after a 350*g* spin for 5 minutes at room temperature. Forty-seven percent of the TF activity was pelleted after centrifuging at 20,000*g* for 30 minutes at 20°C. A negligible difference occurred between the activity pelleted at 50,000*g* and 20,000*g*; however, 76% of the activity was pelleted after centrifugation at 100,000*g*. The pellet obtained from the 100,000*g* spin was examined by electron microscopy and was found to contain vesicles approximately 10 nm in diameter. These data are consistent with TF budding off of the cell surface in vesicles into the culture medium. This extracellular TF may be an important source of active TF in the atherosclerotic plaque and injured vessel wall.

Adjusted Dose Antithrombotic Regimens Eliminate Nitroglycerin-Antithrombotic Interaction. D. A. Vorchheimer, L. Baruch, D. A. Sandler, I. C. Guzman, S. Hellkamp, E. J. Topel, and M. Califf. Mount Sinai School of Medicine, New York, NY; and Duke Clinical Research Institute, Durham, NC.

When intravenous (i.v.) nitroglycerin (NTG) is combined with heparin therapy for patients with acute coronary syndromes, attenuation of anticoagulant effect has been reported: partial thromboplastin time (PTT) values are lower in patients receiving i.v. NTG + heparin (HEP) compared with patients receiving i.v. HEP alone. Because most of these studies did not mandate HEP target dosing schedule, it is unknown whether therapeutic levels of anticoagulation can be achieved using a strict dosing algorithm. It is also unknown whether direct thrombin inhibitors such as Hirudin are affected by concomitant NTG treatment. In GUSTO-IIb, i.v. HEP and hirudin (HIR) were both given according to a strict dosing nomogram. We studied PTT values (sec ± SEM) adjusted for weight in patients selected as to whether they did (NTG+) or did not (NTG−) receive i.v. NTG. No consistent effect of NTG was seen for the study group as a whole (although *$p < 0.05$, NTG+ vs NTG− for day 2 PTT). No consistent effect of NTG was found for either HEP or HIR. However, NTG+ patients required more frequent changes in study drug for both HEP (4.58 vs 4.01) and HIR (2.56 vs 2.10, both $p = 0.0001$), suggesting that although NTG may well interact with antithrombotic treatment, the clinical impact of such an effect can be overcome by rigorous adherence to a dosing nomogram.

	NTG+ ($n = 9281$)	NTG− ($n = 2961$)
Weight (kg)	77.84 ± 0.16	77.46 ± 0.28
Baseline PTT	33.58 ± 0.40	34.51 ± 0.53
6 hour PTT	91.09 ± 2.41	87.85 ± 0.87
12 hour PTT	75.31 ± 0.35	75.26 ± 0.60
24 hour PTT	68.47 ± 0.31	68.42 ± 0.51
Day 2 PTT	68.29 ± 0.29	70.66 ± 0.49*

Emergency Transesophageal Echo in the Diagnosis of Massive Pulmonary Embolism and Response to Therapeutic Intervention. J. Walters, H. Soto, A. Chaudhary, M. Raza, F. Ahmad, and J. Fleischman. Divisions of Cardiology/Pulmonary Medicine, Mount Sinai Services at Queens Hospital Center, Jamaica, NY.

In the United States, 50,000 deaths per year are thought to be a result of pulmonary embolism (PE). In many patients, death results from the failure to make a correct and timely diagnosis of PE. Less frequently, however, a patient with a large PE may present in a hemodynamically unstable state, such that cardiogenic shock and death ensues despite prompt treatment.

To decrease mortality and reduce the ominous complications of unresolved PE, we have demonstrated that in an acute setting, transesophageal echo (TEE) is a useful option. TEE may be used to expeditiously confirm a massive PE that may be responsible for the patient's unstable hemodynamic state. Our patient presented in acute hypoxemic respiratory failure requiring mechanical ventilation. The suspicion for PE in this patient was highly probable, but because his unstable cardiopulmonary state it was decided to forgo a V/Q scan. TEE was used to visualize and confirm the presence of a large thrombus occluding the right pulmonary artery with resulting right ventricular global hypokinesis. Once the thrombus was identified, thrombolytic therapy was initiated based on two criteria: 1) the patient's initial unstable cardiopulmonary state with severe hypoxemia and evidence of right ventricular failure, and 2) large occlusive thrombus. A repeat TEE demonstrated resolution of the responsible thrombus and resolution of right ventricular failure. This case illustrates a promising utility of TEE in evaluating PE and its response to therapeutic intervention.

Clinical Geriatrics

Improving Communications Among Dementia Patients. F. Bocako, B. Krupsky. The Jewish Home and Hospital, Bronx, NY.

To enhance quality of life and promote dignity among patients with dementia, it is necessary to improve their communication skills and social interaction. A multimodal approach established by a speech language pathologist used language and communication techniques to conduct "Breakfast Club" programs for patients with dementia at the Jewish Home and Hospital. Participants helped prepare the meals and congregated at the "kitchen" table where conversation and social interaction were facilitated by the leaders. Groups of five participants interacted for periods of 6 to 24 weeks. Results showed that participants can improve their communication and language skills and renew procedural memories for preparing, sharing, and eating meals. Statistical analyses showed significant improvement in standardized communication, procedural memory, and behavior test scores, for the "Breakfast Club" study group compared with a control group. We conclude that in selected patients with dementia social communication skills can resurface by using a "Breakfast Club" model.

Assessing Survival of Elderly Nursing Home Residents. B. Breuer, S. Wallenstein, C. Feinberg, and L. S. Libow. The Jewish Home and Hospital, New York, NY; and Mount Sinai School of Medicine, New York, NY.

Decisions regarding care of nursing home (NH) residents are often based on the usual life expectancy in one's facility. To facilitate a more individualized approach to their care, we developed a statistical model that predicts survival time of elderly NH residents. The subjects of our retrospective cohort study resided at the Manhattan or Bronx sites of the Jewish Home and Hospital (JHH) from January 1, 1986 through July 1, 1986 ($n = 1145$). We obtained the following data from medical charts or from JHH computerized information: date of birth, ethnicity, religion, vital status, monthly income from Social Security, date of admission, date of discharge, and/or death; morbidities and their extent of impairment, and levels of dependence in activities of daily living as of July 1, 1986. The follow-up period ended on January 17, 1996. We used accelerated failure time models to generate our final model, which predicts the median survival, as well as the 25th and 75th percentiles. The model was derived from a random sample of half of the residents, and was validated on the remaining residents. Multivariate analysis revealed that the following are highly significant predictors of survival: age; sex; extent of impairment of the respiratory, cardiac, neurological, endocrine/metabolic systems; a summary activities of daily living (ADL) score based on transferring, toileting, and eating; and marital status. Surprisingly, our data strongly suggest that the amounts of monthly Social Security payments are inversely related to survival. This model can facilitate the formulation of appropriate public health policies for NH residents.

The Evaluation of Pain in Verbally Disruptive Nursing Home Residents. E. Callahan, M. Lantz, A. Likourezos, T. Castor, and B. Brewer. The Henry L. Schwartz Department of Geriatrics and Adult Development, and Department of Medicine, Mount Sinai School of Medicine, New York, NY; and The Jewish Home and Hospital, New York, NY.

The prevalence of pain among nursing home residents is high, with multiple medical and psychiatric comorbidities creating barriers to assessment and treatment. Surveys suggest that more than 50% of nursing homes residents experience significant pain, with undertreatment a clinical concern. Residents with dementia pose a particular challenge regarding the appropriate evaluation of pain, which may be a contributing factor in syndromes of behavioral disturbance, such as screaming and verbal disruption.

Groups of verbally disruptive residents ($n = 54$), nonverbally disruptive residents ($n = 22$), and residents with recent fractures ($n = 35$) were surveyed regarding type and severity of cognitive impairment, medical diagnoses, medications, and demographics. A modified version of the McGill Pain Inventory, a line-body drawing, and measures of discomfort, agitation, and functional status were used. Primary caregivers were also interviewed regarding their assessment of pain among the residents.

Pain symptoms were identified in all three groups. Residents with recent fractures were more likely to be prescribed acetaminophen ($p = 0.043$) and were the only residents with any orders for narcotic analgesics. Pain among verbally disruptive residents was associated with osteoarthritis ($p = 0.05$), neurologic disease other than stroke ($p = 0.047$), use of antidepressants ($p = 0.077$), and antiparkinson medications ($p = 0.055$). There was a low agreement between the patient and caregiver assessment of pain ($\kappa = 0.300$). Patients with higher mini-mental status examination were more likely to complain of pain and more likely to receive analgesic medications.

Certain types of pain and discomfort are a factor in verbally disruptive behavior. Lack of caregiver/patient agreement of pain and difficulties assessing the type of pain create barriers to treatment. Therapeutic interventions for this vulnerable population must be actively addressed.

Underutilization of Angiotensin-Converting Enzyme Inhibitors in Older Patients after Myocardial Infarction. G. Mendelson and W. S. Aronow. Department of Geriatrics and Adult Development, Mount Sinai School of Medicine, New York, NY.

Angiotensin-converting enzyme (ACE) inhibitors should be administered to older patients after myocardial infarction (MI), who have congestive heart failure (CHF), an anterior wall MI, or a left ventricular ejection fraction (LVEF) $\leq 40\%$, unless there are specific contraindications to their use. A retrospective chart review during 1997 of all patients seen in the Phyllis and Lee Coffey Geriatrics Practice at Mount Sinai Medical Center indicated that 161 patients had an anterior wall MI and no contraindications to ACE inhibitors. The 161 patients included 96 women and 65 men, mean age 82 ± 8 years (range, 65–96 years). Of 161 patients with anterior wall MI, 56 patients (35%) were treated with ACE inhibitors; 45 patients (28%) were treated for CHF. Of 45 patients with anterior wall MI and CHF, 30 patients (67%) were treated with ACE inhibitors. A measurement of LVEF was obtained in 58 (36%) of 161 patients with anterior wall MI. Of these 58 patients, LVEF was $\leq 40\%$ in 44 patients (76%). Of 29 patients with anterior wall MI, CHF, and a LVEF $\leq 40\%$, 24 patients (83%) were treated with ACE inhibitors. Of 15 patients with anterior wall MI, no CHF, and a LVEF $\leq 40\%$, four patients (27%) were treated with ACE inhibitors. These data indicate underutilization of ACE inhibitors in older patients after MI who have CHF, an anterior wall MI, or a LVEF $\leq 40\%$ in an academic hospital-based geriatrics practice.

Underutilization of Beta Blockers in Elderly Patients with Coronary Artery Disease. G. Mendelson and W. S. Aronow. Department of Geriatrics and Adult Development, Mount Sinai School of Medicine, New York, NY.

A retrospective chart review during 1997 of all patients seen in the Phyllis and Lee Coffey Geriatrics Practice at Mount Sinai Medical Center indicated that 233 patients had documented coronary artery disease (CAD). The 233 patients included 149 women and 84 men, mean age, 82 ± 8 years. Prior myocardial infarction (MI) was present in 162 (70%) of 233 patients with CAD. Of 162 patients with prior MI, 38 (23%) were receiving beta blockers. Of 124 patients with MI not receiving beta blockers, 19 (15%) had contraindications to beta blockers. Of 143 patients with prior MI and no contraindications to beta blockers, 38 (27%) were receiving beta blockers. Of 233 patients with CAD, 53 (23%) were receiving beta blockers. Of 180 patients with CAD not receiving beta blockers 34 (19%) had contraindications to beta blockers (severe peripheral vascular disease in 11 patients, hypoglycemic episodes on insulin in 8 patients, bronchospasm in 7 patients, heart failure in 4 patients, bradycardia in 3 patients, and systolic blood pressure less then 100 mm Hg in 1 patient). Of 199 patients with CAD and no contraindications to beta blockers, 53 (27%) were receiving beta blockers. These data indicate underutilization of beta blockers in older persons with prior MI or angina pectoris in an academic hospital-based geriatrics practice.

Partnership Beyond Restraints. R. R. Neufeld, J. M. Dunbar, and L. S. Libow. The Jewish Home and Hospital, New York, NY; and Mount Sinai School of Medicine, New York, NY.

Partnership Beyond Restraints was a collaborative effort, supported by the Health Care Financing Administration (HCFA), to reduce the use of restraints and enhance the quality of life for nursing home residents. In Georgia, Illinois, and Texas, we convened task forces with representatives from all segments of the nursing home community. This is the one of first times that providers, regulators, and family representatives have collaborated toward a common goal. We worked with members of the task forces to co-sponsor educational sessions about how to provide safe, cost-effective alternatives to restraints.

Each task force implemented educational sessions in a different way. For example, in Georgia, the proprietary nursing home association assumed much of the leadership for four large workshops. In Illinois, a subcommittee worked on three train-the-trainer sessions in different regions of the state. In Texas, the provider association sponsored a session at their annual conference and then a subcommittee collaborated on two large workshops.

According to O.S.C.A.R., the federal data bank, restraint rates declined nationally from 21% to 17.5%, from September 1995 until June 1997. Data in O.S.C.A.R. can lag behind by as much as a year. By state, the decreases were as follows: Georgia, 22% to 12.5%; Illinois, 21% to 13.26%; and Texas, 25% to 24%. In addition, we will report on how other variables, provider, government and family/consumer participation, and state size, affected both physical restraint rates.

Training high percentages of both provider staff and surveyors led to the greatest reduction in use of physical restraint, although provider training appears to have been more important. In Illinois, restraint use declined 36%, 58% of facilities sent staff to training and 97% of surveyors were trained. In Georgia, restraint use declined 43%; 53% of all facilities sent staff to training; and 20% of the surveyors received training. In Texas, the restraint rate declined 4%, and 36% of surveyors and 23% of facilities received training.

Long-Term Effects of a Mandatory Fourth Year Medical Student Rotation in Geriatric Medicine. E. Olson, L. Libow, M. Hsu, R. Tideiksaar, and E. Chichin. Department of Geriatrics and Adult Development, Mount Sinai School of Medicine, New York, NY; and The Jewish Home and Hospital, New York, NY.

This study assessed the effect of an obligatory fourth-year medical student rotation in geriatric medicine on subsequent medical training and practice. A questionnaire was sent out in August 1990 to all graduates of the Mount Sinai School of Medicine who had completed the obligatory fourth year medical student rotation in geriatrics that began with the Class of 1984. Data were compiled from 306 responses (53% response rate). The respondents were divided into two subgroups based on their responses: Group 1 those alumni who regularly render care to the frail elderly, and Group II, those who rarely render care to the frail elderly. The groups did not differ demographically except by specialty, with more internists and family practitioners rendering care to older patients. Sixty-two percent of all respondents were still in residency training. More than half the alumni felt prepared to deal with ethical issues (60%), recognize illness in the elderly (54%), and assess functional status (52%). However, the study population as a whole did not feel adequately prepared to deal with arranging home care services and addressing Medicare/Medicaid issues. Group I felt more positively than those in Group II that their geriatric clerkship experience was useful to them in their residency training or current practice. Approximately

70% of all respondents and a similar proportion of those who care for the elderly felt that the length of the geriatric rotation (4 weeks) in medical school should remain the same or be increased. Most respondents in both groups reported that they enjoy caring for older patients (87% and 64%, respectively). Our data reaffirm the need for and the effectiveness of mandatory geriatric education at the medical student level. As physicians who have been exposed to formal geriatric education advance in their careers, their recognition of the need for such education grows.

The Utilization of Postmenopausal Hormone Replacement Therapy in a Community Hospital-Based Ambulatory Clinic. R. P. C. Soriano and M. P. Schori. Englewood Hospital and Medical Center, Englewood, NJ.

Background: Postmenopausal hormone replacement therapy (HRT) generally is underutilized in the ambulatory setting despite the benefits involved with therapy. The main deterring factors are vaginal bleeding and the risks of breast cancer and endometrial cancer. The long-term outcomes of estrogen use, however, include reduction in osteoporosis risk, coronary heart disease, and serum lipid levels, and possibly a delay in the onset of Alzheimer's disease. We therefore studied to determine the prevalence of postmenopausal HRT and the factors associated with its use or nonuse. The current literature regarding HRT utilization as well as risks and benefits was also reviewed.

Design: A retrospective review of 176 clinic charts.

Setting: An ambulatory clinic in a teaching community hospital in northern New Jersey.

Participants: 176 postmenopausal women older than 50 years of age (mean age, 63.5 ± 9.5 years).

Measurements: Current users, past users, and never users of HRT.

Results: Of the 176 women, 19.9% were currently using estrogen, 4.0% had used estrogen, and 76.1% had never used hormones after menopause. The mean duration of current use was 33.2 months, whereas for past users it was 18.6 months. Unlike never users, the current users were more likely to have had natural menopause (odds ratio (OR), 0.25; 95% confidence interval (CI), 0.07–0.91, $p = 0.02$) and to be receiving calcium supplementation/vitamin D (OR = 3.31; 95% CI, 1.04–9.98; $p = 0.03$). There was also a trend for current users to be married but it did not reach statistical significance. HRT was never discussed in 88.0% of the never users as determined by chart review. Sixty percent of the patients of the current senior medical residents were not receiving HRT and were never offered the therapy. Similarly, 15.0% of the patients of the junior medical residents and 11.0% of the patients of the medical interns were never offered HRT.

Conclusion: Postmenopausal HRT is greatly underutilized in the ambulatory setting. Our findings of 19.9% utility is congruent with the low HRT utilization of 17–32% in the general population. Concerns on the part of both the patient and the primary care physician regarding HRT play a significant role in its underutilization. Moreover, the determinants of its utilization are primarily related to socioeconomic factors. Widespread dissemination of information regarding benefits and risks of HRT is needed to allay the questions and fears of the patient and the primary care physician.

Clinical Immunology

***†The Intestinal Epithelial Cell Glycoprotein, gp180, binds to CD8 Sites Distinct from Those of Class I MHC Molecules.** N. A. Campbell, L. Toy, and L. Mayer. Division of Clinical Immunology, Mount Sinai School of Medicine, New York, NY.

Previous studies in our laboratory have indicated that gp180, a novel molecule expressed by intestinal epithelial cells, selectively activates $CD8^+$ suppressor T cells. This 180 kd molecule appears to bind to CD8, activate CD8-associated p56*lck* and appears to be important in suppressor T cell activation by normal intestinal epithelial cells. It has not, however, been directly documented that gp180 binds to CD8 molecules. Thus, we wanted to directly show that GP180 binds to CD8 molecules and identify the CD8 binding regions involved. An enzyme-linked immunosorbent assay was used with gp180 as the capture antigen and CD8-Fc fusion protein as the detection reagent. Binding of gp180 to CD8 was detected using a horseradish peroxidase-conjugated anti-Fc antibody.

Recent studies from other laboratories have documented the site of interaction between CD8 and MHC class I. We were, therefore, interested to determine whether the class I MHC binding portion of the CD8 molecule is involved in its interaction with gp180. Fusion proteins from the α_3 domain of class I MHC molecule (the CD8 binding domain) were used in IEC:T cell cocultures. Cells were incubated with the α_3 fusion protein (or the class II MHC β_2 fusion protein) and washed before being cocultured with intestinal epithelial cells for 0, 1, 2 or 5 minutes. T-cell lysates were run on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel and an anti-phosphotyrosine blot was performed. The α_3 fusion protein did not have any impact on the activation of CD8-associated p56*lck*, suggesting that gp180 interacts with sites on CD8 distinct from class I MHC molecules. This conclusion was confirmed using monoclonal antibodies specific to different epitopes on the CD8 molecule. These antibodies have been characterized previously and identify epitopes involved in the interaction of class I MHC with CD8 (OKT8a, C, I_1, and I_2). A coculture assay and Western blot as previously described were performed. The results indicate that two anti-CD8 antibodies (OKT8B and OKT8F), recognizing non-class I binding sites, inhibit the activation of CD8-associated kinases by intestinal epithelial cells. This may point to critical amino acid sequences in the CD8 molecule that are involved in gp180 interactions and may account for the activation of p56*lck* by gp180 but not by MHC class I.

The Novel CD8 Ligand, gp180, has the Genetic Property of Associating with Nonclassical Class I Molecules. N. A. Campbell,[1] R. Blumberg,[2] S. Balk,[3] M. Kronenberg,[3] and L. Mayer.[1] [1]Division of Clinical Immunology, Mount Sinai School of Medicine, New York, NY; [2]Division of Gastroenterology, Brigham and Women's Hospital, Boston, MA; [3]Division of Oncology, Beth Israel Hospital, Boston, MA; and [4]Molecular Biology Institute, UCLA, Los Angeles, CA.

Previous studies have shown that intestinal epithelial cells (IEC) express a 180 kd glycoprotein, gp180, that binds to CD8, activates CD8-associated p56*lck*, and appears to be important in suppressor T cell activation induced by normal IECs. This molecule is expressed by epithelial cells in the gastrointestinal tract, the thymus, the respiratory tract and by placental syncytiotrophoblast. Since gp180 does not interact with the TCR nor does it activate the TCR-associated fyn kinase, we had previously postulated that nonclassical class I molecules, such as CD1d, might play a role in antigen presentation to T lymphocytes by IECs. Co-immunoprecipitation studies have confirmed our hypothesis that CD1d does indeed associate with gp180. To confirm that CD1d actually binds to gp180, we used an enzyme-linked immunosorbent assay where gp180 was the capture antigen. Varying concentrations of purified CD1d or a CD1d fusion protein were incubated with the gp180 and detected with the mouse anti-human CD1d monoclonal antibody, D5. Binding of gp180 with CD1d was confirmed in this assay.

CD1d is not expressed in other organs where gp180 is seen, such as placental syncytiotrophoblasts. HLA-G is a nonclassical class I molecule expressed by syncytiotrophoblasts. Furthermore, it has been postulated that HLA-G has an immunosuppressive role (nonrejection of the fetus) in the placenta. Thus, we wanted to determine whether or not HLA-G associates with gp180 on the surface of placental syncytiotrophoblasts. Lysate from normal placental tissue was immunoprecipitated with anti-HLA antibody (W6/32) coated beads and an anti-gp180 Western blot was performed. A 180 kd band was detected in the anti-HLA immunoprecipitate but not in the controls. This may indicate a generic role for gp180 to associate with nonclassical class I molecules and allow for the generation of a co-receptor complex. As gp180 binds to and activates CD8-associated p56*lck*, the nonclassical class I molecule (either CD1d or HLA-G) may interact with the TCR, thereby activating TCR-associated fyn kinase. Independent activation of these kinases could then result in the overall activation of the $CD8^+$ suppressor T cell.

Human Recombinant Interleukin-10 Induces T-Cell-Dependent Immunoglobulin Secretion by Resting Human Peripheral B Cells. Y. Kuang and L. Mayer. Division of Clinical Immunology, Mount Sinai School of Medicine, New York, NY.

We have previously shown that interleukin-10 (IL-10) enhanced T-cell-dependent Ig secretion by human B cells induced by anti-CD3 monoclonal antibodies (mAbs) (446). To further investigate the effect of IL-10 on Ig secretion by resting B cells, we cultured purified human peripheral B cells with IL-10 and varying numbers of T cells for 7 days. Supernatants were collected and Ig (IgG, IgM, and IgA) was measured by enzyme-linked immunosorbent assay (ELISA). Although there was minimal Ig secretion in the supernatant of purified B cell cultures alone, Ig secretion was observed in the supernatant of T- and B-cell co-cultures in the presence of IL-10. Ig secretion induced by IL-10 was related to the number of T cells in the co-culture. Ig secretion was observed with as few as 24,000 T cells at a T/B ratio 1:4 and increased as the number of T cells increased. Maximal Ig secretion was noted when B cells were co-cultured with T cells at a ratio of 4:1. IL-10 induced Ig secretion in a concentration-dependent manner. Addition of IL-10 in increasing amounts correlated with increased Ig secretion, ranging from 2- to 3-fold at 12.5 ng/mL IL-10 up to 10- to 100-fold at 200 ng/mL. The effect of IL-10 on B-cell differentiation in the anti-CD3 system appeared to depend on a cognate T cell-B cell interaction. Addition of the anti-CD40 ligand mAb, 5C8, to anti-CD3 mAb (446)-activated T- and B-cell co-cultures completely blocked anti-CD3 mAb-induced Ig secretion, whereas it had minimal effects on IL-10-induced Ig secretion by unactivated T/B co-cultures. These results suggest that IL-10-induced Ig secretion by resting B cells is T-cell-dependent and may be mediated in part by a cognate interaction.

Immunosuppression of Tumor-Bearing Hosts Is Reversed by Tumor Resection. S. Salvadori, G. Nartinelli, and K. Zier. Division of Clinical Immunology, Immunobiology Center, and Department of Surgery, Mount Sinai School of Medicine, New York, NY.

The growth of antigenic tumors in an immunocompetent host suggests that conditions to elicit antitumor immune responses *in vivo* are suboptimal. We have previously reported that cytotoxic T cells (CTL) were demonstrable early after inoculation of CMS5 fibrosarcoma cells into naive mice, but that they disappeared within 3 weeks. At this time, these mice were unable to reject a challenge with parental cells. Such functional nonresponsiveness was accompanied by the loss of signal transduction ability by splenic T cells. In particular, there were reduced levels of $p56^{lck}$, $p59^{fyn}$, and ζ proteins, all of which are critical for signal transduction through the T cell receptor. In addition, there was a decrease in $CD3^+$ T cells and an increase in the number of cells bearing the Mac1 and $Gr1^{bright}$ markers that characterize mature neutrophils. Neutrophils have been shown to suppress T-cell function by a mechanism that involves oxidative stress. Because it is logical to assume that such an environment would make it more difficult to elicit antitumor immune responses via immunotherapy, we asked whether resection of the tumor could reverse these abnormalities. To test this, mice were inoculated s.c. with CMS5 cells. After 28 days the tumors were resected. We observed a decrease in the number of $Mac1/Gr1^{bright}$ cells and an increase in the number of $CD3^+$ cells in the spleen within 24 to 28 hours of tumor resection. By day 10 these values were normal. Levels of $p56^{lck}$ and ζ had returned to normal as well. The functional implications of these changes were illustrated by the reduced growth rate or complete rejection of parental tumor cells in resected, but not naive mice. Our results suggested that tumor resection not only led to the reversal of immune suppression, but also unmasked a population of T cells able to mediate protective immunity *in vivo*.

Reduction in gp180 and CEA Expression in Patients with Crohn Disease. L. S. Toy, X. Y. Yio, and L. Mayer. Divisions of Clinical Immunology and Gastroenterology, Mount Sinai School of Medicine, New York, NY.

We have previously demonstrated that the expression of gp180, a novel intestinal epithelial cell membrane glycoprotein, which appears to mediate T cell-epithelial cell interactions, is reduced in patients with Crohn disease (CD). This reduction appears to be critical to the failure of intestinal epithelial cells (IECs) from patients with CD to activate CD8+ suppressor T cells. Because preliminary studies suggest that gp180 is a unique member of the family of carcinoembryonic antigen (CEA) glycoproteins, we sought to determine whether CEA expression is also altered in CD. IECs were isolated from surgical specimens or colonoscopic biopsies by dispase treatment and then stained with fluorescein isothiocyanate conjugated monoclonal antibodies to gp180 (B9) and the common N-terminus of CEA subfamily members (Col-1). The results were analyzed by flow cytometry. Confirming previous studies, gp180 expression, as manifested by mean fluorescence intensity (MFI) and percent of IECs staining with B9, was lower in patients with CD than in normal controls (mean MFI, normal vs CD, 4.4 vs 1.1). Similarly, a significant decrease in CEA expression was seen in CD IECs when compared with normal IECs (mean MFI, normal vs CD, 4.4 vs 0.6, $p < 0.05$). Thus, both gp180 and CEA expression appears to be altered in CD IECs, which suggests that a global defect may exist in the expression of members of the carcinoembryonic antigen glycoprotein family in patients with CD.

Generation of Anti-tumor Cytotoxic T Cells Depends on a Signal Not Delivered by Tumor Cells, But That Can be Provided in trans. I. Wernick and K. Zier. Division of Clinical Immunology and Immunobiology Center, Mount Sinai School of Medicine, New York, NY.

Considerable evidence suggests that tumor cells poorly activate T cells. For immunotherapy to be an effective treatment modality, it is crucial to understand the mechanisms underlying T-cell unresponsiveness. Previously, we showed that murine CMS5 tumor cells did not stimulate the generation of cytotoxic T cells (CTL) efficiently. Here, we asked whether this was because the tumor cells inhibited the induction of CTL directly. CMS5 tumor cells ($H\text{-}2^d$) and syngeneic spleen cells ($H\text{-}2^d$) were cocultured with allogeneic responder splenocytes ($H\text{-}2^k$). Our results showed that, in contrast to suppression, a synergistic response was observed resulting in greater levels of cytotoxicity on CMS5 than induced by stimulation with either cell alone. Because we had reported previously that T-cell-mediated immunity was elicited by the *in vivo* inoculation of interleukin-2 (IL-2)-secreting tumor cells, we tested whether the availability of IL-2 now enabled the CMS5 cells to stimulate CTL generation directly. In no case was lysis observed by cells from cultures in which splenocytes were stimulated with IL-2-secreting tumor cells alone. To test the effect of allogeneic professional antigen-presenting cells in the culture, naive splenocytes were stimulated with splenocyte tumor cells derived from two different strains. Cultures stimulated with parental CMS5 cells only showed good killing of target cells syngeneic with the spleen cells, but no killing of target cells syngeneic with CMS5. In contrast, in cultures stimulated with splenocytes and IL-2-secreting CMS5 cells form two different strains, there was good killing on targets syngeneic with body splenocytes and tumor cells. These results demonstrated that CMS5 cells did not directly suppress the generation of CTL, that tumor-derived IL-2 was necessary but not sufficient for the induction of CTL, and that spleen cells delivered an essential signal for CTL generation that could be provided in trans.

Dermatology

Differential Expression of P53 and BCL-2 by UVA and UVB: A Novel Mechanism Regulating UV-Induced Apoptosis. Y. Wang, B. Rosenstein, S. Goldwyn, M. Lebwohl, X. Zhang, and H. Wei. Department of Dermatology, Mount Sinai School of Medicine, New York, NY.

The induction of apoptosis by ultraviolet (UV) radiation and other DNA-damaging agents plays a critical role in monitoring the accumulation of genetic damage and the suppression of tumor development. Tumor suppressor gene p53 acts as an inducer of apoptosis and arrest of cell growth, whereas the apoptosis suppressor gene bcl-2 is able to antagonize the induction of apoptosis by p53, but not the growth arrest in G1. However, the action of UVA and UVB on apoptosis has been investigated very little. We hypothesize that UVA and UVB induce apoptosis by modulating balances between p53 and/or bcl-2 genes. Using MCF-7 cells (a human breast cancer cell expressing wild-type p53 and bcl-2) as a model, we demonstrated that UVB significantly induced the expression of p53, but had no effect on the expression of bcl-2. The induction of p53 was dose- and time-dependent with the highest expression at 24 hours after exposure to 2 kJ/m^2 of UVB. On exposure to an equally cytotoxic dose of UVA (150 kJ/m^2), the cells exhibited the opposite gene expression pattern: the expression of p53 was unchanged, but the expression of bcl-2 was down-regulated. Up-regulation of p53 by UVB and down-regulation of bcl-2 by UVA were consistent with the morphological and DNA alterations of apoptosis. Our results suggest that UVA and UVB cause cell damage by different mechanisms and that the balances between the expression of p53 and bcl-2 play an important role in regulating the apoptosis induced by UV exposure.

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Wells Syndrome Precipitated by Arthropod Assault. I. Friedman, A. Sapadin, and J. Baral. Department of Dermatology, Mount Sinai School of Medicine, New York, NY.

Wells syndrome, also known as eosinophilic cellulitis, is an uncommon recurrent dermatitis with eosinophilia. The dermatitis is notable for its resemblance to acute bacterial cellulitis and its recurrent nature. Wells speculated in 1979 that the condition represents a hypersensitivity response to various antigenic stimuli. We report one striking case precipitated by an arthropod assault.

The patient, a 68-year-old African-American woman, was in her usual state of excellent health when she was awakened from her sleep by a millipede bite. At that time, the patient found the insect crawling on the dorsum of her left hand. Several days later, the patient presented to the emergency room complaining of fever, swelling, blisters, and severe pruritus of her hands. The clinical impression at that time was bullous cellulitis involving the dorsa of both hands. The patient was treated with oral cephalexin, Burow's soaks, clobetasol propionate ointment, and diphenhydramine. The patient was referred to the dermatology outpatient clinic where she was seen several days later. At that time, she complained of swelling and pruritus of her feet and noted minimal improvement in her hands. Two weeks later, the patient was seen and hyperpigmented plaques on the hands were noted. The patient than developed pruritic, erythematous patches on her upper arms, buttocks, and knees which evolved into violaceous indurated plaques. A slate-colored, irregularly outlined plaque on the heel of the right foot was also present at the site of previous bullous involvement. Laboratory data at the time of her third examination was significant as follows: platelet count, 568,000 (nl: 150,000–450,000); white blood cell count, 16.5 (nl: 1–10.5); differential: neutrophils, 8.0; basophils, 0.0; lymphocytes, 9.0; monocytes, 2.0; losinophils, 81.0. Histopathologic examination revealed a superficial and deep perivascular dermatitis with numerous interstitial eosinophils and flame figures. Electron microscopic examination demonstrated numerous dense core granules of degranulated eosinophils as well as fragments of eosinophil cytoplasm. The combination of the case history, impressive clinical picture, characteristic histopathologic and electron microscopic findings, and the exclusion of other diseases confirmed the diagnosis of Wells syndrome.

Captopril-triggered Linear IgA Bullous Dermatosis. I. Friedman, A. Sapadin, and D. Rudikoff. Department of Dermatology, Mount Sinai School of Medicine, New York, NY.

Linear IgA bullous dermatosis (LABD) is a subepidermal vesicobullous eruption occurring in adults characterized by a linear pattern of IgA deposition along the cutaneous basement membrane zone. The clinical manifestations are variable and may be confused with dermatitis herpetiformis or bullous pemphigoid. Generally, however, vesicles are arranged in herpetiform, sausage-like, or rosette-like patterns on erythematous or normal-appearing skin. A drug-induced etiology has been reported with lithium, iodine, vancomycin, diclofenac, and amiodarone. A case related to captopril has also been described previously. We report one additional case triggered by this agent.

The patient, a 57-year-old woman, had a long history of hypertension and congestive heart failure. Two weeks after initiating captopril therapy, she presented to the emergency room with a pruritic blistering eruption involving the trunk and extremities. She was hospitalized with a diagnosis of varicella and discharged 3 days later without improvement. Several weeks later, she was referred to the dermatology service for evaluation. Physical examination revealed multiple crusted erosions and intact, tense vesicles. Some were confluent, particularly on the thighs, where arciform, semicircular, and rosette-like arrangements were apparent. There was no intraoral involvement. A biopsy specimen was split for routine histopathology and direct immunofluorescence (DIF). Histopathologic examination revealed a subepidermal cleft with a dense, chronic perivascular infiltrate. DIF demonstrated IgA deposition along the dermal-epidermal junction in a linear pattern, confirming the diagnosis. The condition eventually cleared after treatment with dapsone.

Although it is often difficult to establish a causal relationship between administration of a drug and a subsequent reaction, the temporal relationship with the ingestion of the captopril implicates this angiotensin-converting enzyme inhibitor as the most likely etiologic agent. In the absence of a drug rechallenge, however, it is impossible to state with certainty that the LABD was induced by captopril.

The Effect of Ultraviolet B Phototherapy on HIV-1 RNA Plasma Viral Load: A Self-controlled Prospective Study. J. M. Gelfand,[1] D. Rudikoff,[2] M. Lebwohl,[2] and M. E. Klotman.[3] [1]Harvard Medical School, Boston, MA; and [2]Department of Dermatology and [3]Division of Infectious Diseases, Mount Sinai School of Medicine, New York, NY.

Background: The safety of ultraviolet B (UVB) phototherapy in patients with human immunodeficiency virus (HIV) infection is controversial. Evidence from cell and transgenic animal models suggests that UVB potently up-regulates HIV activity. Nevertheless, HIV+ patients frequently receive UVB phototherapy to treat a variety of skin conditions.

Methods: To determine whether UVB exposure up-regulates HIV activity in humans we studied plasma viral loads in 12 HIV+ patients undergoing UVB phototherapy. Patients were on stable antiviral medications (or no antivirals) for at least 6 weeks, and had no major illness or immunization in the 2 months before starting phototherapy. Patients received treatments at the same time of day, three times per week, for 8 weeks. Viral loads were measured by the Amplicor HIV-1 Monitor™ RT PCR assay at baseline, 2 hours after, and 48 hours after the first treatment of the week, each week, for up to 8 weeks.

Findings: Viral loads showed no significant change (3-fold change from baseline) at early stages of treatment when low doses were used or at later stages when higher doses were used. Only 1 of 12 patients experienced a significant increase in viral load during treatment whereas 2 of 12 patients experienced a significant decrease in viral load and 9 of 12 had no change. Trend analysis showed that most patients had no significant pattern of viral load whereas one patient experienced an increasing trend and three patients had a decreasing trend of viral load. The overall trend indicated no pattern of change in viral load ($p > 0.25$). Of note, three patients had undetectable viral loads (<100 copies/mL) and their viral levels remained undetectable throughout treatment with phototherapy. CD4 counts also remained unchanged (mean pre = 277, mean post 285, $p = 0.67$). All patients experienced clinical improvement in their skin disease during treatment.

Interpretation: Our intensive study of patients with HIV infection who were undergoing phototherapy showed no significant effect of UVB exposure on plasma viral load. UVB phototherapy is highly efficacious in treating many of the skin disorders that dramatically impair the quality of life of patients with HIV.

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The Isoflavone Genistein Inhibits Ultraviolet B-Induced Initiation and Promotion of Photocarcinogenesis via an Antioxidant Mechanism. J. M. Gelfand,[1] X. Zhang,[2] M. Lebwohl,[2] and H. Wei.[2] [1]Harvard Medical School, Boston, MA; and [2]Department of Dermatology, Mount Sinai School of Medicine, New York, NY.

We have previously demonstrated that the isoflavone genistein inhibits chemically induced skin carcinogenesis. In this series of experiments, the photoprotective effects of the isoflavone genistein in SKH-1 hairless mice exposed to ultraviolet B (UVB) radiation were investigated. Tumor formation was evaluated in a two-stage photo-chemical carcinogenesis model. In experiments using UVB as an initiator followed by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) promotion, mice pretreated with topical genistein showed increased tumor latency and showed a 40% decrease in tumor prevalence and a 3.3-fold reduction in tumor burden compared with mice treated with topical vehicle. Similarly, in experiments using UVB as a promoting agent, preceded by initiation with 7,12-dimethylbenz(a)anthracene (DMBA), mice pretreated with topical genistein also showed increased tumor latency and showed a 33% decrease in tumor prevalence and a 4.5-fold reduction in tumor burden compared with mice treated with topical vehicle. Mechanistic studies showed that topical genistein decreases hydrogen peroxide production (H_2O_2), lipid peroxidation and 8-hydroxyldeoxyguanosine formation but has no effect on pyrimidine dimer formation in the skin of hairless mice exposed to acute and chronic UVB. These data suggest that topical genistein inhibits UVB carcinogenesis probably through its antioxidant properties.

Successful Treatment of Acute Cutaneous Lupus Erythematous with Isotretinoin. C. Inch, A. Sapadin, L. Friedman, D. Rudikoff and M. Gordon. Department of Dermatology, Mount Sinai School of Medicine, New York, NY.

Several reports have described successful treatment of subacute or discoid chronic cutaneous lupus erythematosis with oral retinoids. We describe a case of acute cutaneous lupus erythematosis (LE), clinically consistent with the bullous variety, successfully treated with isotretinoin. Our patient, a 31-year-old woman, had an 11-year history of LE that was variably controlled with multiple medications. She had been hospitalized 6 months previously for a toxic epidermal necrolysis-like flare that did not respond to treatment with corticosteroids, quinacrine, and dapsone. Transfer to the burn unit was necessary at that time. A pulse dose of cyclophosphamide resulted in significant improvement but was complicated by the development of myelosuppression. The administration of azathioprine was discontinued for the same reason. Isotretinoin (80 mg/day) was prescribed to avoid the systemic toxicities of these agents. The patient's skin remained clear for the next 7 weeks while she received isotretinoin and low-dose corticosteroids. The most recent admission was prompted by a seizure at which time the isotretinoin was inadvertently discontinued. Nine days later, cutaneous erosions reappeared on the upper back. These progressed to include involvement of the scalp, face, chest, and left arm. Isotretinoin treatment was reinitiated and resulted in rapid clinical improvement. This case emphasizes the use of isotretinoin to induce remission in a patient with acute cutaneous LE who is refractory to conventional therapies. Because the use of oral contraceptives in LE patients is discouraged by some authorities, adequate counseling regarding alternative forms of birth control is a prerequisite to initiating retinoid therapy in these patients.

Cutaneous Extravascular Granuloma as a Manifestation of Systemic Immune Complex Disease. E. Lowenstein,[1] C. Gropper,[1] D. Frankel,[1] and R. Phelps.[2] Departments of [1]Dermatology and [2]Dermatoplasty, Mount Sinai School of Medicine, New York, NY and Bronx VA Medical Center, NY.

An 89-year-old Greek man with a medical history significant for essential thrombocythemia, chronic anemia, and ulcerative colitis presented 3 years ago with an eruption on the hands and face whose resolution correlated temporally with the discontinuation of sulfasalazine. Biopsy at the time demonstrated a dense mixed cellular infiltrate, medium vessel vasculitis with adjacent noncaseating granuloma.

The patient recently presented with an acute eruption of erythematous tender nodules on the face, ears, and neck which extended over the course of a week to involve the arms, hands, and thighs. He complained of generalized fatigue, chills, and abdominal discomfort with occasional vomiting, but no diarrhea. The patient was found to be febrile and anemic and admitted for transfusion and further evaluation.

Laboratory studies demonstrated anemia, mild thrombocythemia, and negative serology for syphilis (RPR), lupus erythematosis (ANA), Wegener granulomatosis (C-ANCA, P-ANCA), and rheumatoid arthritis (RF). Multiple skin biopsies demonstrated extravascular necrobiotic granulomas involving leukocytoclastic vasculitis of neighboring vessels as well.

Ulcerative colitis, and less frequently, myelodysplasia have been reported in association with cutaneous necrobiotic granulomas, more recently referred to as Winkelmann granuloma or palisaded neutrophilic and granulomatous dermatitis. This most likely represents an evolving necrobiotic granuloma in association with systemic vascular immune complex disease and may represent a dermatologic clue to an underlying systemic illness.

Half a Century Old Case of Chronic Osteomyelitis. E. Lowenstein and A. Lefkowits. Department of Dermatology, Mount Sinai School of Medicine, New York, NY.

A 93-year-old man with diabetes was admitted for progressive left hip pain which hindered his ambulation. The Department of Dermatology was called to evaluate an asymptomatic lesion on his right shin which was noted during the course of his admission physical. The patient stated that the injury to his right shin was sustained while jumping from one car to another on a train in World War II. There was no history of broken bones or any other significant impairment secondary to the injury. The lesion "healed" over many years without any oral antibiotics or other medical attention other than dry dressings. The lesion had been stable for many years. He had no complaints of pain or other symptoms secondary to the lesion.

The patient is an extraordinarily alert and well-appearing 93-year-old, incredulous of the great attention his leg has attracted. Examination demonstrated a broad hyperpigmented patch on the right shin with a large focal sinus channel and adjacent mounds of hyperkeratosis and powdery crusts, which were positive for hyphae on KOH smear. No erythema, edema, tenderness, or exudate was noted. His feet demonstrated prominent generalized scale with associated onychodystrophy.

Radiological evaluation of the right tibia demonstrated a sinus tract from the skin to the distal tibia, marked expansion of the tibia with patchy sclerosis of the medullary cavity (sequestrum), and circumferential cortical thickening (involucrum). No local bone destruction or soft tissue mass or swelling was noted. Bone scan demonstrated abnormal thickening of the tibia with diffuse increased activity. This picture is most consistent with chronic osteomyelitis.

This case demonstrates the benign and indolent nature of chronic osteomyelitis treated with conservative management.

Eruption of HIV Seroconversion. A. Sapadin,[1] J. Gelfand,[2] L. Friedman,[3] J. Gumprecht,[4] M. Gordon,[1] and D. Rudikoff.[1] [1]Department of Dermatology and [4]Division of Infectious Diseases, Mount Sinai School of Medicine, New York, NY; [2]Harvard Medical School, Boston, MA; and [3]George Washington University School of Medicine, Washington, DC.

An acute retroviral syndrome occurs in approximately 50% of individuals 1–8 weeks after exposure to the HIV virus. Recognition of the mucocutaneous manifestations, when present, facilitates prompt diagnosis and treatment. We report the case of a 34-year-old homosexual man who tested negative for antibodies to the human immunodeficiency virus (HIV) 3 weeks before hospitalization for an acute illness that included high fevers, malaise, sore throat, and a mucocutaneous eruption. Cutaneous findings consisted of severe seborrheic dermatitis and an exanthem characterized by multiple erythmatous macules and papules, some with a hemorrhagic center, distributed on the trunk and proximal extremities. Palmar involvement was subtle, but present. The mouth contained an enanthem with palatal petechiae and a well circumscribed erosion. Lymphadenopathy was notably absent. Routine laboratory testing revealed a pancytopenia (white blood cell count, 2,600; platelets, 90,000; hemoglobin 11 g/dL), lymphopenia (0.7 [normal range 1.0–4.5 × 1000]), and an elevated partial thromboplastin time. A rapid plasma reagin test was nonreactive. An erythematous papule on the chest was biopsied and sent for pathologic examination. Hematoxylin and eosin stain revealed a mild interface dermatitis, whereas special stains failed to reveal microorganisms. Testing for HIV DNA by polymerase chain reaction was positive. Shortly thereafter, seroconversion to HIV antibody positivity occurred. Triple antiretroviral therapy was promptly initiated. The aim of this presentation is to facilitate recognition of the eruption of HIV seroconversion. The authors hope that this will lead to earlier identification, treatment and counseling of infected persons.

Patients Infected with the Immunodeficiency Virus. A. Sapadin,[1] J. Gelfand,[2] K. Howe,[3] L. Friedman,[4] and D. Rudikoff.[4] Departments of Dermatology, [1]Mount Sinai School of Medicine and [2]Beth Israel Medical Center, New York, NY; [3]Harvard Medical School, Boston, MA; and [4]George Washington University School of Medicine, Washington, DC.

Human immunodeficiency virus (HIV) disease is characterized by a gradual decline in immune function manifested by a sequence of opportunistic infections and malignancies. It is clear that Kaposi's sarcoma, undifferentiated non-Hodgkin's B-cell lymphoma, primary central nervous system lymphoma, and anorectal squamous cell carcinoma occur with increased incidence in HIV disease. Associations with other malignancies such as nonmelanoma skin cancer, Hodgkin's lymphoma, and T-cell lymphomas, although not conclusively proven, have also been described. We report the occurrence of dermatofibrosarcoma protuberans (DFSP) in two patients with HIV infection, an association not reported previously, to the best of our knowledge.

The first patient was a 50-year-old HIV-positive man with a history of multiple basal cell carcinomas (BCC). He developed several asymptomatic papules within a scar located on the upper back. The scar was a result of prior surgery for the treatment of a BCC. Histopathologic examination of the biopsy specimen demonstrated a DFSP adjacent to a BCC. The lesion was excised, but recurred. It was then treated by Mohs micrographic surgery, at which time the tumor was found to have peripheral and deep extension beyond clinically recognizable borders. The second patient, a 36-year-old HIV-positive woman, developed a large nodular DFSP in the left lateral infraclavicular area.

HIV-associated neoplasias are generally more aggressive, respond poorly to treatment, and are associated with a higher morbidity and mortality than the same tumors in non-HIV-infected individuals. Although comment regarding a causal relationship between HIV and DFSP is premature, the report of this rare entity in association with HIV disease is noteworthy.

Paraneoplastic Pemphigus. A. Sapadin,[1] N. Said-Al-Naief,[2] H. Lummerman,[2] L. Friedman,[3] and R. G. Phelps.[1] Departments of [1]Dermatology and [2]Oral and Maxofacial and ENT Pathology, Mount Sinai School of Medicine, New York, NY; and [3]George Washington University School of Medicine, Washington, DC.

Paraneoplastic pemphigus is a recently described autoimmune mucocutaneous syndrome characterized by refractory and severe stomatitis, polymorphous cutaneous lesions, and characteristic immunopathologic findings in the context of a known or occult neoplasm.

Chronic lymphocytic leukemia was diagnosed in our patient, a 54-year-old Indian man, in January 1995. Later that year, he began to experience painful mouth ulcerations and hoarseness. He was subsequently hospitalized several times for recurrent and refractory stomatitis. In September, 1996 he developed a cellulitis of the left wrist at a prior i.v. site. A bulla formed and the lesion gradually evolved into a large verrucous plaque. The intraoral pain became progressively more severe and the patient was transferred to our institution for the investigation and treatment of refractory stomatitis and dysphagia. Physical examination was notable for diffuse erosions of the lips, tongue, palate, and buccal mucosa. The skin findings were significant for a large verrucous plaque on the distal left forearm. Generalized lymphadenopathy was present.

Multiple viral cultures were negative. Endoscopic examination revealed a panesophagitis and multiple esophageal ulcerations. Generalized lymphadenopathy was demonstrated on a whole-body computed tomography scan with massive lymph nodes noted in the retroperitoneal area. A biopsy of the buccal mucosa revealed suprabasilar cleft formation with a tombstone pattern of basilar keratinocytes and acantholysis. Although the direct immunofluorescence testing was only faintly positive, indirect immunofluorescence testing was positive on monkey esophagus at a titer of 1:640 and on rat bladder at a titer of 1:160. A high molecular weight complex of four polypeptides (250, 230, 210, 190 kD) was immunoprecipitated, confirming the diagnosis of paraneoplastic pemphigus. The patient experienced marked improvement on combination therapy with prednisone and cyclosporine. Paraneoplastic pemphigus should be suspected in any patient with a severe and refractory stomatitis in the context of a known or suspected neoplasm.

Antisense Suppression of Interleukin-6 Gene Expression in Psoriatic Skin Fibroblasts. R. M. van Solingen,[1] R. S. Panush,[1] and L. R. Espinoza.[2] [1]St. Barnabus Medical Center, Livingston, NJ; and [2]Louisiana State University Medical Center, New Orleans, LA.

Psoriasis (Ps) and psoriatic arthritis (PsA) are not uncommon. We found that unstimulated psoriatic skin, synovial fibroblasts, and abnormal fibroblasts constitutively produce significant interleukin (IL)-6 messenger RNA. This finding suggests that IL-6 may be important in the pathogenesis of Ps and PsA. In psoriatic skin fibroblasts treated with cyclosporine A (CsA) and dexamethasone (DEX), the IL-6 mRNA is reduced compared with control untreated cells. We concluded that CsA and DEX may act at the transcriptional level of IL-6.

Purpose. We sought to inhibit the IL-6 mRNA in psoriatic skin fibroblasts with an oligonucleotide fragment antisense to the 5′-end of the IL-6 mRNA.

Methods. Fibroblasts were isolated by 3-mm skin-punch biopsy from patients with PsA and culture. The antisense oligonucleotide and lipofectin diluted in serum-free media were combined, added to 40 to 50% confluent cells and incubated for 20 hours. Total RNA was extracted and transcribed into single-stranded cDNA which was then amplified by polymerase chain reaction with primers for IL-6 and β-actin.

Results. The antisense oligonucleotide specific for IL-6 mRNA suppressed the mRNA expression for IL-6.

Conclusion. Oligonucleotides antisense to the mRNA of IL-6 can be used to block IL-6 mRNA expression even more efficiently than CsA and DEX. This suggests the possibility of exciting new treatments for the inflammatory and proliferative processes which characterize psoriasis and psoriatic arthritis and related conditions.

Isoflavone Genistein Protects UVB Radiation-induced Photodamage in Hairless Mouse and Human Skin. H. Wei, R. Phelps, X. Zhang, J. Gelfand, Y. Wang, and M. Lebwohl. Department of Dermatology, Mount Sinai School of Medicine, New York, NY.

We have investigated the effect of genistein, an isoflavone from soybeans, on ultraviolet (UV) radiation-induced damage to mouse and human skin. In current studies, we have examined the effects of genistein on UVB-induced short-term effect such as erythema in mouse and human skin and protooncogene expression in mouse skin, as well as long-term effects such as skin wrinkles and histological changes in the epidermis and dermis of hairless mice. Topical application of genistein before UVB irradiation prevented acute erythema and blocked UVB-induced expression of protooncogenes c-*fos* and c-*jun* expression in mouse skin. Chronic exposure of mouse skin to UVB (thrice weekly at a dose of 90 mJ/cm^2 for 10 weeks) caused substantial photodamage to mouse skin characterized by wrinkles and histological alterations, including epidermal hyperplasia, reactive acanthosis with nuclear atypia, heliodermatitis, and increased number, tortuosity, and complexity of elastic fiber. However, application of genistein before UVB substantially modulated these UVB-induced alterations. In studies of human subjects, topical vehicle alone had no blocking effect on skin erythema (mean effective dose [MED] of 30 mJ/cm^2) compared with the direct UVB radiation. However, topical genistein (5 μmo/cm^2 of skin) 60 min before UV exposure completely blocked skin erythema by all UVB doses ranging from 30 to 90 mJ/cm^2. Further study on the second subject showed that genistein inhibited UV-induced erythema (MED of 45 mJ/cm^2) with a minimal effective dose of 0.1 μmol/cm^2 skin. Our results suggest that genistein may be a promising agent in the prevention of UV-induced skin photodamage.

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Emergency Medicine

Primary Care Physician Study. S. Jacobson. Emergency Medicine, Mount Sinai School of Medicine, New York, NY.

The most pervasive and serious threat to the health care of the Nation is the 40 million uninsured or underinsured individuals in this country. In addition, there has been a rapid growth of newly hired, upwardly mobile individuals who are interested in obtaining health care on demand, when they need and want it. In fact, some may view the primary physician as the gate keeper who prevents them from receiving timely, convenient services, leading them to undervalue the traditional continuity-of-care paradigm. The uninsured worker or unemployed individual does not regularly receive preventative health services nor afford elective surgery. Conversely, the young, upwardly mobile, employed, and insured individual usually has access to preventative health services, but is either unaware of the current standards of adult preventative care or has simply has not established personal priorities to regularly receive these services.

A voluntary and anonymous questionnaire will be administered in Spanish and English by the emergency department (ED) faculty, staff, and volunteer research assistants to 500 patients, their family members, accompanying friends or support persons in the adult and pediatric ED waiting rooms. The study seeks to determine the health beliefs of the individuals using or accompanying patients to the Mount Sinai Hospital (ED), with reference to the value of a personal primary physician and to ascertain the relative values that the study groups give to episodic care via the ED, compared with continuity of care via a primary physician. The study seeks to understand their baseline knowledge and plans (if any) for receiving preventative health care services and to collect vital demographic information on the participants to proactively plan and improve health care delivery systems in the ED.

We assert that it is cost affective to provide episodic primary care through the ED and its associated walk-in-clinic, and that most healthy, employed, and insured individuals would prefer this care on demand, available 24 hours a day. The working poor would also benefit from the ED's resources, if the charges for this care accurately reflected the marginal costs of this care.

Endocrinology

Interleukin-6 Levels Are Not Increased in Women with Postpartum Thyroid Dysfunction. I. Ahmad, E. Diamond, and A. Stagnaro-Green. Division of Endocrinology and Metabolism, Mount Sinai School of Medicine, New York, NY.

Postpartum thyroid dysfunction (PPTD) is an autoimmune mediated thyroid destructive process. Human interleukin 6 (IL-6) is a pleiotropic cytokine which is increased in subacute thyroiditis, amiodarone-induced thyrotoxicosis, Graves' disease and other thyroid-destructive processes. We therefore postulate that IL-6 levels would increase in women with PPTD. In a previous prospective study, we demonstrated that PPTD occurred in 25% (7 of 28) of women with type I diabetes mellitus. The degree of PPTD was mild in the seven women. IL-6 determinations were made on the frozen serum samples of the 28 women during each trimester of their pregnancy and at 1.5, 3, 6, 9, and 12 months postpartum. Serum IL-6 concentration was determined by an enzyme-linked immunosorbent assay (R and D Systems, Minneapolis, MN; range, 0.156–10 pg/mL). IL-6 levels were similar in women with PPTD compared with women without PPTD (mean, 3.06 ± 2.25 vs 2.51 ± 2.21 pg/mL; $p = 0.15$). No difference in the mean IL-6 levels for all 28 women was found between the ante- and the postpartum periods (mean, 2.67 ± 1.82 vs 3.04 ± 2.44 pg/mL; $p = 0.30$). There was no correlation between IL-6 levels and trimester or month postpartum for the 28 women ($r = 0.016$). In conclusion, IL-6 levels during pregnancy or postpartum were not found to be significantly different in women with PPTD compared with women without PPTD. This may be caused by the mild degree of severity of PPTD found in our patient population.

Androgen Receptor Immunoreactivity in Specific Neural Regions in Normal and Hypogonadal Mice. S. Apostolinas, G. Rajendren, A. Dobrjansky, and M. J. Gibson. Division of Endocrinology and Metabolism, Mount Sinai School of Medicine, New York, NY.

In addition to peripheral actions, testicular androgens have effects in the central nervous system on reproductive function and behavior. These activities are mediated via androgen receptors. The present study evaluated the effects of testicular androgens on the distribution of androgen receptors in both normal and hypogonadal male mice. The reproductive system in untreated hypogonadal mice remains infantile, because of a deletion in the gene for gonadatropin-releasing hormone, and the adult males are infertile and fail to show normal male sexual behavior. Groups of adult normal mice were left intact, castrated, or castrated and given subcutaneous capsules containing testosterone propionate (TP). Hypogonadal mice were intact or given a TP capsule. One week later, all mice were deeply anesthetized and perfused. Their brains were processed for immunocytochemistry using an antibody for androgen receptors (rabbit polyclonal, Vector Labs). As in other species, androgen receptor immunoreactivity was largely confined to neuronal nuclei. The numbers of immunoreactive neurons were counted in defined regions implicated in male reproductive behavior, including the lateral septum (LS), preoptic area (POA), bed nucleus of the stria terminalis (BNST), ventromedial hypothalamus (VMH), and medial amygdala (MA). There were significantly fewer labeled neurons in every region of the brains studied except the VMH of hypogonadal mice than in intact normal mice, but TP treatment of the hypogonadal males induced normal numbers of neurons with androgen receptor immunoreactivity in each of these regions. Castration resulted in a dramatic decline in androgen receptor immunoreactivity in normal male LS, POA, and BNST, and TP treatment reversed this effect in the POA and BNST. These results suggest that the deficiency in androgen receptors in regions critical for male behavior in hypogonadal mice is secondary to their undeveloped testes, and that normalization is readily achieved in the presence of circulating testosterone. Plasticity was also observed in normal males with just 1 week of castration and TP replacement.

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How Important Are Common Immunoregulatory Genes to Susceptibility to Graves' Disease? G. Barbesino,[1] Y. Tomer,[1] M. Keddache,[2] E. S. Concepcion,[1] D. A. Greenberg,[2] and T. F. Davies.[1] [1]Division of Endocrinology and Metabolism and [2]Department of Psychiatry, Mount Sinai School of Medicine, New York, NY.

Graves' disease (GD) is a thyroid autoimmune disease inherited in a complex fashion. For this reason, identification of susceptibility genes necessary for the disease has been difficult. Several immunoregulatory genes have been implicated by association studies, including: CTLA-4, a recently described protein involved in antigen presentation, located on chromosome 2q33, the T-cell receptor α (TCRA) and β (TCRB) gene complexes, located on 14q11 and 7q35, respectively, and the immunoglobulin gene complex (IgH), located on 14q32. We have used a polymorphic microsatellite marker located within the gene or gene complex for each of these candidates. This allowed us to assume a fixed recombination fraction of 0.01 for classical linkage analysis. Sixty subjects from 9 multiplex families were studied, with 17 affected subjects. Using this set of families, we have previously shown evidence of linkage with two loci on 14q24.3-31 (GD-1 and GD-2), with a maximum LOD score of 2.1 at a penetrance of 80% and with a dominant mode of inheritance. Genotyping was performed with an ABI Prism 310 and linkage was analyzed using LIPED software. The set of data was not informative enough to accept or reject linkage with any of the candidates with a recessive mode of inheritance. The LOD scores obtained assuming a dominant mode of inheritance were:

Gene	CTLA-4		TCRA		TCRB		IgH	
Penetrance %	30	80	30	80	30	80	30	80
LOD score	−1.337	−1.467	−1.635	−1.943	−0.205	−1.325	−1.182	−2.122

Our results showed evidence against linkage with all the candidate genes, given the consistently negative LOD scores obtained. Unlike association studies, these linkage analyses should have allowed the identification of major contributors to GD susceptibility. The data, therefore, show that the immunoregulatory genes studied are not major contributors to the etiology of GD.

Post-translational Processing of the Thyroidal Thyrotropin Receptor Identification of a Cleavage Site Near Residue 366. P. N. Graves, H. Vlase, and T. F. Davies. Department of Medicine, Mount Sinai School of Medicine, New York, NY.

Detergent extracts from thyroid plasma membranes contain different forms of the soluble thyrotropin receptor (TSHR), some with an intact protein backbone (holoreceptors) and others derived by apparent cleavage of the backbone into two major fragments (α and β subunits). Binding of TSHR peptide polyclonal antibodies and epitope-mapped murine monoclonal antibodies (mAbs) to these fragments has been informative regarding the potential cleavage site(s). For example, Ban et al. (*Endocrinology* 1992; 131) showed that an antibody to TSHR 352–366 bound only holoreceptor and α subunits, and we showed recently that TSHR 397–415 antibody bound only holoreceptor and β subunits (*Endocrinology* 1996; 137). These data suggested cleavage between TSHR residues 366–397. In the present study, we have used a new panel ($n = 10$) of murine mAbs generated against highly purified recombinant TSHR 22–415 to further refine this area of cleavage. These new mAbs bound intact 90-kDa TSH holoreceptor together with either the α (50 kDa) or β (35 kDa) TSHR subunit on immunoblots of human thyroid plasma membranes. However, one mAb (M5) bound recombinant hTSHR 22–415 and thyroidal holoreceptors but did not bind to either α or β subunits. In an enzyme-linked immunosorbent assay (ELISA) screen for binding to 26 overlapping peptides spanning TSHR 22–415, M5 bound only peptide TSHR 352–371. The failure of M5 to recognize either subunit as a result of holoreceptor cleavage suggested the loss of the M5 epitope. This may have occurred secondary to direct cleavage of the epitope or excision of a fragment containing the epitope in the TSHR 352–371 region. Alternatively, receptor processing may have involved two steps using cleavage near the epitope followed by further peptide trimming by an exopeptidase. Taken together, the results obtained with a variety of epitope-defined antibodies suggest a cleavage site in the region of TSHR 352–371 and close to residue 366.

MUCI Expression in Prostate Carcinoma: Correlation with Clinical Grade and Stage. A. Kirschenbaum, J.-P. Wang, M. Eliashvili, S. H. Itzkowitz, and A. C. Levine. Department of Endocrinology, Mount Sinai School of Medicine, New York, NY.

Introduction and Objectives: Mucins have been implicated in the biological behavior and progression of several types of cancer. The aims of this study were to define the expression pattern of one particular mucin, MUC1, in benign and malignant human prostate tissue and to determine whether MUC1 expression correlates with tumor grade and stage.

Methods: Immunohistochemical staining for MUC1 expression using an antiMUC1 monoclonal antibody (B27.29, Biomira, Edmonton, CA) was performed on four fetal prostates, 4 BPH specimens and 38 radical prostatectomy specimens (prostate cancer).

Results: In human fetal and BPH specimens, there is an apical pattern of expression of MUC1 in epithelial cells, similar to that reported in other benign tissues. A high percentage of prostate cancer specimens (58%) demonstrated a diffuse, cytoplasmic MUC1 staining pattern. There is a clear correlation between diffuse MUC1 staining and Gleason pattern (G2–5):

	G2	G3	G4	G5
Diffuse/total	1/11 (9%)	16/25 (64%)	4/5 (80%)	4/4 (100%)

The table below demonstrates that there is also a strong correlation between %diffuse MUC1 staining and final TNM pathologic stage.

	T2	T3	N1
Diffuse/total	3/14 (21%)	15/20 (75%)	2/3 (67%)

Conclusions: These data indicate that: 1) MUC1 expression is prevalent in prostate cancer (92% of cases were MUC1-positive); 2) diffuse cytoplasmic MUC1 staining correlates with advanced Gleason pattern and advanced pathologic stage.

Induction of Hyperthyroid Graves' Disease in H-2^k Mice. M. Kita,[1] L. Ahmad,[1] H. Vlase,[1] P. Unger,[2] P. N. Graves,[1] and T. F. Davies.[1] [1]Division of Endocrinology and Metabolism and [2]Departments of Medicine and Pathology, Mount Sinai School of Medicine, New York, NY.

Graves' disease is an autoimmune thyroid disease characterized by the presence of autoreactive T cells and autoantibodies specific for the thyrotropin receptor (TSHR). Autoantibodies to the TSHR act as TSH agonists and stimulate the thyroid to cause thyroid hypertrophy and hyperthyroidism. No animal models exist for Graves' disease. Using either human or murine recombinant TSHR preparations, we have previously failed to induce hyperthyroid disease in mice and have only induced TSHR autoantibodies which blocked TSH action. However, a recent report (Proc. Natl. Acad. Sci. USA 1996; 93:11074) suggested a new approach which we have now confirmed.

We transfected a murine L cell (fibroblast) line with full-length human TSHR cDNA. The L cell line that we used spontaneously expressed a hybrid gene containing A^k and A^d of the murine major histocompatibility complex (MHC) class II molecule (line RT 4.15HP) (kindly provided by R. N. Germain, NIAID, NIH). Flow cytometric analysis showed hTSHR transfected and nontransfected RT 4.15HP cells expressed comparable levels of MHC class II molecules on their cell surface. The transfected cells but not untransfected cells expressed the hTSHR in a functional array, exhibiting TSH-increased stimulation of cyclic AMP generation. We then immunized AKR/N (H-2^k) mice by administering intraperitoneally every 2 weeks either hTSHR transfected cells or with untransfected RT 4.15HP cells as control. Mice were bled 12 weeks after the first immunization and their thyroid hormone levels were measured. Three of 18 mice immunized with hTSHR transfected RT 4.15HP showed markedly increased serum T3 and T4 hormone levels compared with controls, with the highest thyroid hormone levels being T3, 419.3 ng/dL (normal <70), and T4, 16.46 μg/dL (normal <4), and each exhibited weight loss and hyperactivity. We had sacrificed six mice at the time of writing, including two hyperthyroid animals, and found histological features of thyroid hyperactivity in both hyperthyroid animals. These changes included thyroid enlargement, thyroid cell hypertrophy, and colloid droplet formation, all consistent with Graves' disease. None of the control mice showed these features.

These results demonstrate that immunization with naturally expressed human TSHR in mammalian cells, in the context of MHC class II expression, can induce functional TSHR autoantibodies that stimulate the mouse thyroid gland. This protocol should serve as a useful procedure for examining the immune and metabolic consequences, as well as the immunogenetics, of hyperthyroid Graves' disease.

A Quality Improvement Initiative for Patients with Diabetes Undergoing Hemodialysis. C. J. Levy, E. Smith, C. Schechter, J. Winston, and S. H. Roman. Department of Endocrinology, Mount Sinai School of Medicine, New York, NY.

Morbidity and mortality (M&M) for patients with diabetes (DM) receiving hemodialysis (HD) is almost twice as high as HD patients without DM. Unlike other parameters of effective uremic care for patients receiving HD, such as nutritional status, control of BP, and adequacy of dialysis, there are few data on the proportions of patients in the dialysis setting whose diabetes care meets the American Diabetes Association (ADA) recommendations. Surveys in nondialysis patients have shown that most patients with DM are not receiving care as recommended by the ADA Clinical Practice Recommendations. We studied the processes of DM care provided to patients at two HD sites to assess whether their DM care met ADA standards. Of 191 patients undergoing HD at the two sites, the 54 subjects with DM underwent chart review (CHTRV). Charts were reviewed for sociodemographic data and documentation of defined processes of care during the previous year. The mean age of the patients was 60 ± 11.5 years (mean ± SD), with a DM duration of 19.6 ± 9.1 years, a duration of dialysis of 2.6 ± 2.4 years, and BMI of 26.4 ± 6.3 kg/m^2. There were 26 (48%) African American, 23 (43%) Hispanic, 4 (7%) Caucasian, and 1 Pakistani patient. Twenty-eight patients were treated with insulin (INS) and 26 did not presently require insulin (NINS). Twenty-two (41%) of 54 patients had documentation of dilated eye examinations noted in the previous year. Twenty-seven patients (50%) had documentation of previously existing diabetic eye disease. Of these 27 patients, 16 with preexisting eye disease had documentation of a dilated examination within the year. A total of 16 HBA1C measurements were performed within the year with a mean value of 8.5 ± 2.9% (range, 4.6–15.6). Nine (32%) of 28 INS patients had mean HBA1C levels of 10.1 ± 2.6%, and 7 (27%) of 26 NINS had mean HBA1C levels of 6.4 ± 1.38 (p = 0.02). Nine (17%) of the 54 patients (all INS patients) were noted to perform home glucose monitoring (HGM). Complete lipid profiles were performed in 15 (28%) of 54 patients in the previous year. Of 29 patients with documented CAD, 5 had lipid profiles. Fifteen (28%) of the 54 patients had documented foot examinations in the previous 6 months. Seven (37%) of 19 patients with documented PVD had foot examinations performed. In conclusion, like most DM patients, patients with DM on HD may not be receiving the level of care recommended by the ADA despite their long duration of diabetes and high degree of disease severity. A quality improvement initiative which includes patient and staff education about DM and assessment of barriers to care is indicated. Furthermore, DM patients receiving HD may benefit from case management with participation of an endocrinologist. A prospective study is necessary to determine the impact of such an initiative on outcome.

Human Thyroid Follicle Formation in Simulated Microgravity. A. Martin,[1] A. Zhou,[1] R. Gordon,[2] and T. F. Davies.[1] Departments of [1]Medicine and [2]Pathology, Mount Sinai School of Medicine, New York, NY.

We used simulated microgravity as provided in a NASA-developed bioreactor to generate artificial human thyroid tissues containing multiple thyroid follicles. We used a diluted (3% v/v) suspension of extracellular matrix (ECM; basement membrane extract, Matrigel^R) in combination with recombinant human keratinocyte growth factor (KGF, 7 ng/mL) in serum-free medium to support the survival and differentiation of human thyrocytes, leading to morphologically fully differentiated thyroid follicles during a 2-week culture period. Many aggregates of thyrocytes were observed within 6–16 hours under these conditions; in the absence of KGF, aggregation was unremarkable or absent. Thyroid follicles were located in close proximity to each other and had a polarized appearance, expressing microvilli on the luminal side and desmosomes between cells. Immunogold labeling using a primary antibody to human thyroglobulin showed staining of follicular lumina and secretory vesicles, and increasing levels of human thyroglobulin were detected in the culture media. In summary, culture under simulated microgravity allowed direct visualization of thyrocyte/ECM interaction. In addition, these findings demonstrated that TSH was not a requirement for thyroid follicle formation by adult human thyrocytes. Such artificial human thyroid tissues resembled natural thyroid tissue and should have applications for the study of human thyroid disease.

Glomerular Filtration Rate (GFR) Fails to Increase after Chronic Protein Ingestion in Growth Hormone-Deficient Adults. I. Sachmechi, R. Chaiken, M. Eckert-Norton, and H. E. Lebovitz. Division of Endocrinology, Mount Sinai Services at Queens Hospital Center, Jamaica, NY and SUNY Health Sciences Center, Brooklyn, NY.

Growth hormone (GH) levels increase after high protein intake and amino acid infusion. GH infusion for several days in healthy humans increases GFR. Subjects with poorly controlled insulin-dependent diabetes mellitus (IDDM) have increased GFR associated with elevated GH levels. The mechanism by which protein ingestion causes an increase of GFR is currently unknown. GFR failed to increase after acute protein ingestion in GH-deficient adults. We studied the changes in GFR in response to chronic protein ingestion in GH-deficient and control subjects.

We studied five chronically GH-deficient adults and a group of four non-GH-deficient control adults. GFR was measured after 2 weeks on a low-protein diet (0.5 g/kg b.wt./day), and 2 weeks on a high protein diet (3 g/kg b.wt./day). GFR was measured by using the constant infusion (^{125}I-iothalamate) technique, and was corrected for body surface area (1.73 m^2). Compliance with the diet was determined by measurement of 24-hour urinary nitrogen. In the GH-deficient subjects, mean GFR was 105.80 ± 22.26 SD on a low-protein diet and did not change (101.81 ± 22.83 SD) on a high-protein diet. In the control subjects, mean GFR was 131.73 ± 19.28 SD on a low-protein diet and increased to 159.81 ± 17.15 SD on a high-protein diet ($p = 0.08$).

We conclude that chronic adaptation to a high-protein diet is associated with an increase in GFR that might require the presence of GH.

Role of the Estrogen Receptor Gene and the X Chromosome in the Genetic Susceptibility to Autoimmune Thyroid Disease. Y. Tomer,[1] G. Barbesino,[1] E. S. Concepcion,[1] M. Keddache,[2] D. A. Greenberg,[2] and T. F. Davies.[1] [1]Division of Endocrinology and [2]Department of Psychiatry, Mount Sinai School of Medicine, New York, NY.

Autoimmune thyroid disease (AITD) [Hashimoto's thyroiditis (HT) and Graves' disease (GD)] are organ-specific autoimmune diseases with a well-recognized genetic influence. Although polymorphisms in HLA and non-HLA genes have been associated with these diseases, the identification of the necessary genes has been elusive. Because a higher prevalence of both GD and HT is observed in females than in males, the possibility of an X-linked inheritance has been suggested. Sex steroids have also been involved in experimental models, suggesting a role in disease susceptibility. To identify the possible presence of predisposing genes on the X chromosome, we have studied 24 multiplex families with GD or HT or both (149 total and 58 affected members) in a linkage analysis of a panel of 14 polymorphic microsatellite markers, spaced approximately 10 cm apart and spanning the entire length of the X chromosome. We also studied the erythrocyte sedimentation rate (ESR) marker, linked to the α-estrogen receptor gene on chromosome 6q25. Microsatellite markers were amplified using fluorescent-labeled specific primer and polymerase chain reaction (PCR) products were run on an ABI310 automated sequencer followed by semiautomated genotype attribution. Classical linkage analysis was then performed with LIPED, considering AITD, GD, or HT as the "affected" phenotype in three different analyses. Consistently negative LOD scores were observed with all markers when the AITD and GD phenotype were analyzed, in both the dominant and recessive mode of inheritance. When the HT phenotype was studied, only one marker gave a maximized LOD score of 1.4 when assuming a dominant mode of inheritance and a penetrance of 80% (DXS1106), whereas all the other markers gave negative results. A candidate gene approach was used in the analysis of ESR, allowing the assumption of a recombination fraction of 0.01. The results excluded linkage with the AITD phenotype down to a penetrance of 20% in the recessive model and of 99% in the dominant model, respectively. These data suggest the absence of major genetic determinants for HT, GD, and AITD on the X chromosome, rule out a role for variations in the estrogen receptor gene, and support experimental evidence of a predominant role of sex hormone in determining the sex-related differences in the prevalence of AITD.

Development of Monoclonal Antibodies That Inhibit Thyrotropin (TSH) Binding and Block TSH Stimulation. H. Vlase,[1] Y. Bobovnikova,[1] M. Weiss,[1] T. Moran,[2] P. N. Graves,[1] and T. F. Davies.[1] [1]Division of Endocrinology and Metabolism and [2]Departments of Medicine and Microbiology, Mount Sinai School of Medicine, New York, NY.

We have characterized 10 monoclonal antibodies (mAbs) to recombinant murine thyrotropin (TSH) receptor extracellular domain (mTSHR-ecd). Affinity-purified mTSHR-ecd (amino acids 22–415), expressed in a baculovirus-insect cell system, was refolded *in vitro* and used to hyperimmunize female Balb/c mice. Spleens were removed 10 days after a final boost of 25 μg mTSHR-ecd i.p. and i.v., and the cells were fused to SP-2 cells and cloned. Hybridoma supernatants were screened by enzyme-linked immunosorbent assay (ELISA) with folded mTSHR-ecd antigen. Ten of 18 higher affinity hybridomas were selected at random and ascites fluids prepared. Nine of the mAbs were of IgG 1 isotype, and one was IgM. Five mAbs (M3, M4, M5, M6 and M9) inhibited the binding of ^{125}I-TSH to functional hTSHR expressed on Chinese hamster ovary cells, and four (M1, M3, M5, and M9) blocked the TSH-stimulated generation of cAMP, using the same cells. The remaining mAbs appeared to be neutral in their interaction with native TSHR. The mAbs were also compared for their reactivity with mTSHR-ecd under folding (ELISA) and unfolding (reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis) conditions. Most mAbs demonstrated reactivity to both conformational (folded) and linear (unfolded) forms of mTSHR-ecd, suggesting that they were generated primarily against linear epitopes. Four of these mAbs also bound to immunoblots of the natural TSHR α-subunit. However, two mAbs (M4 and M10) showed high affinity for only folded antigen, indicating a preference for conformational epitopes. Mapping the mAb epitopes using 26 overlapping peptides spanning the human (h)TSHR-ecd showed that six bound peptide 397–415, one bound peptide 352–371, one peptide 22–41, and two bound none of the peptides, presumably because they were mouse sequence specific or because their epitopes were split using this peptide design. These epitope-mapped mAbs to the mTSHR-ecd, both receptor blocking and receptor neutral, are reminiscent of blocking hTSHR-Abs found in patients with autoimmune thyroid disease, support the polyclonal theory of hTSHR-Abs, and provide further insight into the structure-function of the TSHR ectodomain.

Inactivation of the p16 Gene in Human Pituitary Tumors by Gene Methylation Is Accompanied by Activation of DNA-Methyltransferase Gene Expression. M. Woloschak,[1] A. Yu,[1] and K. D. Post.[2] [1]Division of Endocrinology and [2]Department of Neurosurgery Mount Sinai School of Medicine, New York, NY.

Methylation of CpG dinucleotides interferes with gene transcription and represents an important mechanism of gene silencing. Methylation of tumor suppressor genes is thought to play a role in early stages of tumor development, because methylation frequently is detected in benign precursor lesions and decreases DNA stability. Our laboratory has demonstrated that the p16 tumor suppressor gene is inactivated in human pituitary tumors and that in most pituitary tumors this inactivation occurs by p16 gene methylation. The hypermethylation of tumor suppressor genes in tumors and tumor-derived cell lines has been linked most consistently to increases in expression and activity of DNA-methyltransferase (MTase), the enzyme which catalyzes DNA methylation. Although increased expression of DNA-MTase in some tumors has been shown to reflect the increased proliferative activity of tumor cells, strong evidence exists that other mechanisms are responsible for high DNA-MTase expression in other tumor cell systems. To further investigate the process of DNA methylation in pituitary tumors, we have studied the expression of the gene-encoding DNA-MTase. Equal amounts of RNA prepared from 18 human pituitary tumors and 5 normal postmortem pituitary specimens were subjected to reverse transcription using oligo-dT and polymerase chain reaction using primers specific for the human DNA-MTase gene. For all samples, GAPDH-specific primers were used to demonstrate equal amounts of intact RNA. In addition, levels of DNA-MTase mRNA were normalized to mRNA levels of proliferating cell nuclear antigen (PCNA) to assess whether increased levels of DNA-MTase mRNA in these tumors might be attributed to increased proliferative activity of these tumor cells. Our results demonstrate that DNA-MTase gene expression is highly activated in human pituitary tumors and that this increased expression occurs beyond what might be attributable to the increased proliferative activity of pituitary tumor cells, suggesting that a novel mechanism underlies the activated expression of this gene in human pituitary tumors.

Gastroenterology

Massive Hepatomegaly Caused by Combined Hydatid Disease and Recurrent Renal Cell Carcinoma. G. Abdelsayed, W. Baddoura, and A. Jarrad. Division of Gastroenterology, Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

Hydatid disease is endemic in areas of the Mediterranean basin. We describe an extremely unusual case of combined hydatid disease of liver and renal cell carcinoma, the latter recurring 12 years after the initial resection and presenting 9 years later with massive hepatomegaly. A 49-year-old Jordanian woman living in Jordan had a localized right renal cell carcinoma in 1975 treated by nephrectomy. In 1987, she presented with abdominal pain and a slowly growing mass in the right upper quadrant. A computed tomography (CT) then showed liver cysts and multiple liver lesions; biopsy of the lesions in 1987 showed a poorly differentiated neoplasm consistent with renal cell carcinoma, and the cyst was diagnosed as hydatid disease. No specific treatment was given. Repeat CT and biopsy showed similar findings in 1992. Recently, she presented to us with progressive abdominal swelling, weakness, and diffuse abdominal discomfort. Pelvic examination was remarkable for a huge liver occupying the entire abdomen with an irregular surface, thrill and bruit, and dilated veins were noted over the abdomen and back. CT scan showed a calcified large cyst in the right lobe and a smaller one in the left lobe with a massively enlarged liver extending to the pelvis and pushing all viscera to the left. Nuclear scan showed a heterogenous vascular tumor. Magnetic resonance imaging showed findings similar to the CT. The portal vein was patent. On angiography, the tumor was supplied from the celiac axis, superior mesenteric artery, and inferior phrenic artery. CTs of the head, neck, and chest were unremarkable; colonoscopy and esophagogastroduodenoscopy were negative. Multiple biopsies from the right lobe showed the same findings as the 1987 and 1992 biopsies done in Jordan, which were available for review. Aspiration of the left lobe cyst was unremarkable. α-Fetoprotein, hepatitis B and C serology were negative. This patient will be treated with chemoembolization to shrink the tumor before surgery. We present this case because of combined pathology, massive hepatomegaly, the very protracted and indolent course, and recurrence of renal cell carcinoma limited to the liver.

Esophageal Intramural Pseudodiverticulosis. E. Y. Baghal, R. Spira, and J. DePasquale. Division of Gastroenterology, Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ; and St. Michael's Medical Center, Newark, NJ.

Esophageal intramural pseudodiverticulosis is a rare disorder of the esophagus in which numerous reticuli form in the wall of the esophagus. We describe a case and review the literature. A 52-year-old black woman with a history of insulin-dependent diabetes mellitus (IDDM) and hypothyroidism presented with intermittent dysphagia of 6 months duration associated with pyrosis and odynophagia, but no regurgitation or weight loss. Laboratory studies showed iron deficiency anemia. Esophagogram showed 1- to 3-mm flask-shaped diverticula scattered along the mucosal wall. Upper endoscopy showed a proximal esophageal stricture with multiple small diverticuli throughout the entire esophagus. The patient responded very well to dilation and antireflux therapy. Esophageal intramural pseudodiverticulosis is a rare condition; only about 60 cases are reported in the literature. Usually patients are older than 50 years when the diagnosis is made. The usual presenting symptom is dysphagia with or without chest pain. The etiology is unclear; however, it appears that stasis and inflammation causing cystic dilation of adnexal glands with subsequent squamous metaplasia plays a role in its development. An increase in intraluminal pressure may also play a role. Esophageal candidiasis has been reported in 50% of cases, but its role in pathogenesis is still speculative. Esophageal strictures and cervical webs are reported in 70 to 90% of cases as well as diabetes mellitus, which is seen in 32% of patients. Treatment is dilation of the stricture and antireflux therapy for relief of symptoms. Esophageal intramural pseudodiverticulosis is a rare cause of dysphagia, which is treated effectively with endoscopic dilation.

Cyclosporine-Induced Nephrotoxicity in Patients with Inflammatory Bowel Disease. U. Mahadevan, A. A. Kornbluth, E. Goldstein, J. George, S. Lichtiger, and D. H. Present. Mount Sinai School of Medicine, New York, NY.

Introduction: Cyclosporine (CS) is a rapid and effective treatment for inflammatory bowel disease (IBD) with a significant toxicity profile, including effects on renal function. The objective of this study is to determine whether the known short-term nephrotoxocity is permanent and/or progressive after a 6- to 9-month course of CS is completed.

Methods: We conducted a retrospective chart review of 99 patients, [53 Crohn's disease (CD), 46 ulcerative colitis (UC)] treated for 6 to 9 consecutive months with CS. The creatinine clearance (Ccr) was calculated before initiating treatment, at consecutive periods during therapy, and at the time of last follow-up (LFU). A regression analysis was performed to determine whether age or mean 6-month plasma CS level played a significant role in the decline in Ccr. The effect of disease type (UC vs Crohn's), sex, hypertension, and concomitant use of 5-ASA agents was analyzed by a student's *t*-test.

Results: Patients were followed up for an average of 3.1 years after beginning a 6- to 9-month course of CS therapy. The mean Ccr and mean decrease in Ccr from baseline levels are shown below:

	Ccr (mL/min)	Mean ↓ Ccr	*p* value
Baseline	109.2	—	—
Month 1	100.1	9.0	0.0001
Month 3	99.8	9.3	0.0001
Month 6	93.0	16.2	0.0001
Month 9	92.4	16.8	0.0001
Month 12	97.8	11.4	0.0001
LFU	93.7	15.5	0.0001

There is a statistically significant decrease in Ccr at each follow-up interval compared with baseline; however, there was no further decline in Ccr once CS was discontinued. Twenty (20%) of 99 patients sustained a >33% decline in Ccr from baseline at LFU. By regression analysis, the mean 6-month plasma CS level was the only independent variable significantly associated with a decline in Ccr.

Conclusions: There is a mild but irreversible decline in CCr in IBD patients treated with CS for 6 to 9 months, but there is no progressive deterioration of renal function once the CS is discontinued.

The Place for Methotrexate in the Treatment of Refractory Crohn's Disease. U. Mahadevan, J. F. Marion, and D. H. Present. Division of Gastroenterology, Department of Medicine, Mount Sinai School of Medicine, New York, NY.

Introduction: The role of methotrexate (MTX) in the treatment of Crohn's Disease (CD) has yet to be defined. Previous studies have shown a response ranging from 39% to 80%. This study attempts to determine the efficacy and role of MTX in the treatment of refractory CD.

Methods and Patient Description: We conducted a retrospective review of 33 patients with refractory CD treated in one practice in which azathioprine (6-MP) had failed in 28 (85%) of 33 patients, cyclosporine had failed in 7 (21%) of 33 patients, and 20 (61%) of 33 patients were receiving chronic steroids. MTX was initially administered and maintained in most patients as a weekly i.m. dose of 25 mg. Several patients were switched to oral MTX. We determined the frequency of response, maintenance of remission, closure of fistulas, reduction of steroid dosage, and drug toxicity. Four patients received two courses of MTX (37 total courses).

Results: Clinical remission was seen in 23 (62%) of 37 courses of treatment. Of 28 prior 6-MP failures, 18 (64%) responded to MTX. In 16 patients with fistulas, complete closure occurred in 4 (25%) and significant improvement in 5 (31%) with an overall fistula response of 56%. Of the 23 responders, 16 (70%) relapsed and 6 (26%) remain in long-term remission with 1 case lost to follow-up. Relapse occurred in 7 (44%) of 16 patients after stopping MTX and 9 (56%) of 16 patients relapsed while receiving MTX. Steroids could be discontinued in 86% of the MTX responders. However, relapse was noted in 50% within 6 months of discontinuation of steroids. Severe toxicity was observed in 2 (6%) of 33 patients. One patient developed severe interstitial pulmonary disease and one liver biopsy documented hepatic fibrosis.

Conclusions: MTX is effective in 62% of patients with chronic CD, in 64% of whom 6-MP had failed. Fistulous response is observed in 56% (closure in 25%). However, relapse is frequent, usually after steroid withdrawal, with only 26% long-term remission obtained. MTX is indicated only after a trial with 6-MP, probably as an interim therapy, with low expectation of long-term response.

An Aberrant Right Subclavian Artery: An Uncommon Cause for Dysphagia. S. M. Schlachter and W. J. Baddoura. Division of Gastroenterology, Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

The most common congenital anomaly of the aortic arch is an aberrant right subclavian artery. This artery arises from the aortic arch and crosses the mediastinum from left to right assuming a retroesophageal position. This may cause compression of the proximal esophagus posteriorly. Dysphagia lusoria (dysphagia by "freak of nature"), however, is an uncommon cause for dysphagia. A 55-year-old woman presented with complaints of solid food dysphagia for 3 months. She denied any history of pyrosis, gastroesophageal reflux disease, weight loss, nausea, vomiting or chest pain. She had no other medical problems. The initial investigation used a barium swallow, which was suggestive of a proximal esophageal lesion, probably of extrinsic nature. Thoracic computed tomography scan with oral contrast did not demonstrate any extrinsic lesion. At endoscopy, the esophageal mucosa was normal; however, there was a shelf-like compression of the posterior wall of the esophagus at 18 cm which easily allowed passage of the scope. Magnetic resonance imaging of the thoracic inlet revealed an aberrant right subclavian artery originating from the posterior aspect of the aortic arch, crossing from left to right and compressing the esophagus. This study confirmed the diagnosis of dysphagia lusoria. Dietary modification improved the patient's symptoms. An aberrant right subclavian artery may be the most common congenital anomaly of the aortic arch; however, this anomaly is an uncommon cause for dysphagia and rarely produces symptoms. Dysphagia lusoria should be suspected in patients with solid-food dysphagia when evaluation suggests extrinsic proximal esophageal compression. This disorder may be managed conservatively with dietary modification, or surgically if needed.

A Solitary Peutz-Jeghers-Type Hamartomatous Polyp of Jejunum Presenting with Gastrointestinal Bleeding and Intussusception: A Case Report. T. K. Shalini, G. Abdelsayed, and C. Anselmi. Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

A 20-year-old Hispanic woman presented with massive lower gastrointestinal bleeding and a hemoglobin level of 5.5. Routine workup including colonoscopy, esophagogastroduodenoscopy, bleeding scan, and angiography was negative. A small bowel barium series revealed suspicion of an intussuscepted mass in the proximal jejunum; no other abnormalities were found. Enteroscopy showed a solitary pedunculated friable exophytic, hamartomatous Peutz-Jeghers-type polyp measuring 3.5 × 2.5 × 2.5 cm with central ulceration in the proximal jejunum. The surface of the pedicle consisted of smooth, glistening, pink-tan mucosa and the base extended into the submucosa, with a cylindrical firm area averaging 0.6 cm in diameter extending into the muscularis of the small bowel along its antimesenteric aspect. The patient did not manifest cutaneous pigmentation typical of Peutz-Jeghers syndrome and gave no family history of the same. Although bleeding occurs commonly in Peutz-Jeghers syndrome, solitary polypoid intussusception occurs rarely. Although this rare condition has been reported previously (three cases) in Asian and Middle Eastern subjects, this is the first known case occurring in the United States.

Early Discharge after Acute Gastrointestinal Bleeding. M. Vijay, M. P. Schori, and W. Klein. Englewood Hospital and Medical Center, Englewood, NJ.

Objective: 1) To risk-stratify patients with acute upper gastrointestinal bleeding into low-risk and high-risk groups for risk of rebleeding and/or death; 2) relationship of risk score and length of hospital stay; 3) relationship of length of hospital stay to interval between admission and endoscopy.

Background: Acute upper gastrointestinal bleeding is a common cause of hospital admission and has been considered as an obligatory admission until the risk of rebleeding has minimized. This means that some patients at low risk stay longer in the hospital, especially when diagnostic endoscopy is delayed. We attempted to identify patients who had a low risk of bleeding and/or death and could be discharged early without adversely affecting outcome. A consensus statement, which was published in the Journal of the American Society of Gastroenterology in March 1986, stated that upper gastrointestinal endoscopy should be strongly considered in all patients with acute gastrointestinal bleeding. If identification of the bleeding source seems appropriate, endoscopy is most accurately done within 24 hours of the bleeding episode. Longstreth and Feitelberg showed the efficacy and safety of outpatient care of selected patients with acute nonvariceal upper gastrointestinal bleeding who underwent endoscopy. They established a policy of early referral to a gastroenterologist along with rapid laboratory investigation and endoscopy with substantial cost saving. Forrest et al., in a prospective trial, showed that the patients who underwent endoscopy within 48 hours of admission, who had lesions which were actively bleeding or which showed evidence of having bled recently in 72% of cases, had success in identifying the site of bleeding which decreased rapidly from 78% within 24 hours of admission to 32% after 48 hours. The chances of seeing the lesion actually bleeding are best in the first 24 hours.

Setting: Englewood Hospital and Medical Center, a community Hospital in Bergen County, NJ.

Participation: Patients admitted to Englewood Hospital from March 1996 to March 1997 with the diagnosis of acute upper gastrointestinal bleeding.

Design: Retrospective chart review of 121 patients with acute upper gastrointestinal bleeding.

Methods: We used a validated scoring system based on age (score 0–2), presence of shock (0–2), comorbidity (0–3), diagnosis (0–2); and endoscopic stigmata of recent hemorrhage (0–2); the maximum possible score was 11 points. The greater the points the higher the risk of rebleeding and/or death. This analysis was based on 95 patients who underwent endoscopy and 26 patients who did not have endoscopy.

Findings: Forty-one (43.15%) of 95 patients scored 4 points or less. Of these patients two (4.9%) rebled and none died. Low risk score identifies patients at low risk for rebleeding and/or death and higher scores identify patients at higher risk. The median hospital stay was 4 days; the duration of hospital stay increased with risk score. Within higher risk score stratification, there was an inconsistent trend toward increasing length of hospital stay as the time between admission and endoscopy increased.

Conclusion: We were unable to find a consistent relationship between early endoscopy and length of hospital stay in those patients with acute upper gastrointestinal bleeding.

General Medicine

Attitudes of Medical Attending Physicians at Elmhurst Hospital Center Toward Legalization of Physician-Assisted Suicide. J. Abando. Department of Medicine, Elmhurst Hospital Center, NY.

Physician-assisted suicide is currently under discussion in the medical profession and in society as a whole. The U.S. Supreme Court recently upheld a New York State law prohibiting physician-assisted suicide. No studies previously had been done of physicians' attitudes toward physician-assisted suicide in an inner-city public hospital serving a multiethnic population, so a survey was conducted by the Elmhurst Hospital Center. A questionnaire used in a previous study (Backman et al. N. Engl. J. Med. 1996; 334:303–309) was distributed to attending physicians. Questions covered demographic characteristics and beliefs, attitudes, and reported experiences with physician-assisted suicide. Odds ratios were calculated and χ^2 analyses were performed for categorical variables. Completed questionnaires were returned by 44 (78.6%) of 56 physicians eligible for the study: 43.2% would ban physician-assisted suicide, 38.6% would support legalization, and 18.2% were uncertain. If physician-assisted suicide were legal, 34.1% would participate, with Ambulatory Care Attendings (50%) more willing to participate than the Subspeciality Attendings (20%; OR = 2.4, $\chi^2 = 4.13, p = 0.04$). Physician-assisted suicide had the least support among the strongly religious ($\chi^2 = 8.22, p = 0.04$). About half of the Medical Attendings surveyed at Elmhurst Hospital Center oppose legalization of physician-assisted suicide, and almost two thirds would not participate in physician-assisted suicide.

Effects of Cross-Cultural Physician-Patient Relationships on Medical Treatment: 1997. M. Desir and R. A. David. Division of General Medicine, Mount Sinai School of Medicine, New York, NY.

Objective: Cultural and language differences have an impact on the delivery of health care. We studied these differences and showed how they affect adherence to medical treatment.

Methods: An evaluative instrument was developed in English and Spanish as a 10-minute survey. Institutional review board approval was obtained. The survey was distributed at Internal Medicine Associates (IMA), a multidisciplinary primary ambulatory medicine site located at the Mount Sinai Medical Center.

Results: The survey completion rate was 86%. Thirteen of the 15 patients who declined participation stated that distrust of the "medical establishment" was their reason, based on racial issues. For categorical data, χ^2 analysis was used. 38 ± 11% ($p < 0.05$) of patients indicated that their physician considered their unique cultural needs; 61 ± 11% ($p < 0.05$) indicated that their physician considered their emotional needs; 44 ± 11% ($p < 0.05$) of patients indicated that family advice played a role in their taking their medication; 28 ± 11% ($p < 0.05$) indicated that their religion or a healer played a role; 73 ± 11% ($p < 0.05$) of patients used prayer as alternative treatment; 25 ± 11% ($p < 0.05$) used herbs, plants, or oils; fifty-two percent of women and 26% of men responded that they would be better understood if their physician was of their same gender, and 25% of women and 11% of men indicated they would be more likely to follow treatment regimens if their physician were of their same gender.

Conclusions. Clear communication leading to trust in the physician is an essential ingredient for patients' decision-making regarding adherence to medical therapy. It is disturbing to find that some patients verbally indicated their distrust for the "medical establishment" and for that reason declined participation in the study. However, it is important to note that even if differences in culture exist, most patients indicated that effort to acknowledge this issue can help bridge the gap. Herbs, plants, oils, religion, and prayer rank among treatments in which patients participate but did not notify their physician. It is important for physicians to be aware of the fact that patients frequently do not share information about nonprescription treatments. More female than male patients indicated that sharing the same gender with their physician improved their communication. This finding is consistent with other studies in which there was no culture or language barrier. It is known to have a negative impact on completion rates of mammograms and Pap smears.

Case Report: Adult Respiratory Distress Syndrome as an Uncommon Complication of Verapamil Overdose. M. Eid, G. G. Abdelsayed, and R. Saporito. Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

A 31-year-old man with a history of variant angina and schizo-affective disorder was evaluated at our Emergency Room 5 hours after ingesting 30 tablets of Verapamil SR 240 mg (total dose, 7200 mg). On admission, the patient was alert, awake, and lethargic. Vital signs were: blood pressure, 60/20 mm Hg; respiratory rate, 20 breaths/min; pulse, 50 beats/min. The pupils were equal, round, and sluggishly reactive to light. Chest x-ray initially revealed right-sided infiltrates which eventually progressed to bilateral infiltrates. The patient remained hypoxemic with Po_2 of 48 mm Hg despite an Fio_2 of 100%. A two-dimensional transthoracic echocardiogram done 2 hours after admission revealed normal left ventricular function and mild dilation of the right ventricle. The Swan-Ganz reading was: pulmonary artery pressure, 31/16 mm Hg; capillary wedge pressure, 16 mm Hg; central venous pressure, 12 mm Hg; mean arterial pressure, 61 mm Hg; cardiac output, 5.01 L/min; cardiac index, 2.69 L/min^2; systemic venous resistance, 814 dyne/s/cm^{-5}. Peak verapamil level was 925 ng/mL on the day of admission. The patient was resuscitated with fluid, dopamine, norepinephrine, and calcium chloride. A temporary pacemaker was inserted and the patient was started on ventilator support. Broad-spectrum antibiotics were started because of admission for possible aspiration pneumonia. The patient remained hypotensive (systolic blood pressure, 60–70 mm Hg) for 5 hours. Aramine was added, after which the patient's systolic blood pressure stabilized at about 100 mm Hg. The patient remained in a state of respiratory distress for 4 days requiring mechanical ventilation with a high Fio_2 (70–90%). Infectious causes were ruled out based on negative tracheal cultures, blood cultures, and the absence of fever or leukocytosis. Verapamil may cause adult respiratory distress syndrome (ARDS) via its effect on inflammatory mediators, fibroblast morphology, extracellular matrix protein synthesis and by its smooth muscle-relaxing effect. Verapamil may also increase capillary perfusion and ventilation-perfusion mismatch. ARDS may be an unrecognized complication of verapamil overdose.

Smoking Cessation Survey at Elmhurst Hospital Center: 1996–1997. S. A. Elalaoui and A. Lyman. Department of Medicine, Elmhurst Hospital Center, NY.

Surveys find that at any time, only 12% to 20% of smokers are actively preparing to stop smoking. Our survey was conducted to determine the distribution of Medical Primary Care Clinic (MPC) current smokers into stages of the transtheoretical model of health behavioral change and to determine how gender, country of origin, educational level, and brand of cigarettes smoked are associated with the stage. We conducted a cross-sectional study of MPC patients using a convenience sample. One of us (SAE) interviewed 62 smokers during the months of August and September 1996. Odds ratios were calculated and χ^2 analyses were performed for categorical variables. Distribution by stage was concentrated in the first two stages: 48% in the precontemplation stage; 38% in the contemplation stage; 12% in the preparation stage. A greater percentage of females were in the precontemplation stage (53%) and a greater percentage of males were in the contemplation stage (44%), but this was not statistically significant. The distribution by age showed a greater percentage of older smokers (65 and older) in the precontemplation stage (62%) and a greater percentage of younger smokers (25–44 years) in the preparation stage (20%, p = NS). American-born smokers tended to have a greater percentage in the precontemplation stage than non-American-born smokers, but this may have been confounded by age. The educational level did not influence the distribution in our sample. Marlboro was smoked by 46% of our patients. The pattern of stage distribution has important implications for the design of interventions to help patients quit smoking. Although gender, age, and country of origin were differentially distributed by stage, these differences did not reach statistical significance. Because patients may cycle between stages, prospective studies with larger samples are needed to determine interventions that are matched to the patients' stages in the transtheoretical model.

Cigarette Advertising Identification and Smoking Behavior in Inner-City Adolescents. A. Lyman and C. Schechter. Department of Medicine, Mount Sinai Services at Elmhurst Hospital Center, NY.

Cigarette advertising creates demand among adolescents; inner-city adolescents are characterized by demographic and behavioral factors associated with a high risk of future smoking. The neighborhoods in which they live contain many billboards advertising cigarettes. However, the impact of advertising in ethnically diverse adolescent populations has not been widely studied. A survey including advertising identification was conducted in 1989 among 662 students in grades 6 through 9 in public schools in the Lower East Side and Chinatown sections of Manhattan. The rate of ever-smoking was 49%; only 15% of students identified themselves as smokers. Smoking status was predicted by a high identification score of cigarette ads, age, living with a smoking sibling(s), and having a smoking best friend. The two most commonly recognized brands were each identified by 78% of the respondents. A relative risk of smoking of 1.24 was incurred for each additional advertisement recognized. Identification of cigarette advertisements was significantly associated in multivariate analysis with age, female gender, self-identification of bi/multiracial ancestry, recall of having seen ads on transportation, in print media and stores; and with the highest category of cigarette consumption. This study demonstrates that inner-city adolescents retain information from cigarette advertisements.

Breast Cancer Screening at Elmhurst Hospital: Provider Knowledge and Reported Behaviors. L. Notorio and A. Lyman. Department of Medicine, Mount Sinai Services at Elmhurst Hospital Center (EHC), NY; and Auerbach S. Health Resources and Services Administration, PHS.

Adherence by primary care physicians to screening guidelines varies (Griffith and Williams. Primary Care 1992; 19:535–556). Guidelines on screening for breast cancer (SBC) from governmental, professional, and voluntary health organizations differ, most notably with respect to women aged 40 to 49 years. Recently, the National Cancer Institute reversed its position and recommended mammogram screening for this age group; the American Cancer Society changed its recommendation to support an interval frequency of 1 year for such screening (Marwick. JAMA 1997; 277:1181). Physicians may be confused by or mistrustful of conflicting recommendations, so a survey was conducted among medical attending physicians at EHC to assess knowledge, attitudes, beliefs, and reported behaviors concerning SBC using a 69-item questionnaire. Demographic variables included: age, gender, years of practice, subspeciality training, additional degrees, and having had continuing medical education (CME) on SBC in the past 2 years. Anonymity was assured to participants, with identifiers used only to determine response. A second questionnaire was sent to nonrespondents. Frequencies were determined and χ^2 or Fisher's exact test analyses were performed for categorical variables using EpiInfo. Thirty-seven questionnaires were distributed: 25 (68%) providers responded. Fourteen (56%) were male; the most frequent age group was 40 to 44 years old (33%). Twelve (48%) had subspeciality training, but twenty (80%) were practicing generalists. Seventeen (68%) reported having had CME within the past 2 years. Twenty-four (96%) order screening mammograms (SM), 17% would order a SM for a 35-year-old woman with average risk, 83% would order a SM for a 40-year-old with average risk, and 79% would order a SM for a 75-year-old with average risk. Ninety-six percent would order a SM for a 35-year-old patient with a family history of premenopausal breast cancer; the same proportion would order a SM for a 45-year-old menopausal patient. Ninety-six percent perform clinical breast examinations; 52% start at varying ages ≤30 years and 16% start at 40 years. Eight-eight percent report teaching breast self-examination; 64% start at varying ages ≤30 years; 8% start at 40 years. Of those who do not teach breast self-examination, the most frequent reason for this was a belief that benefit had not been demonstrated. Eighteen (72%) were aware of recent changes in the guidelines on SBC; 13 (68%) of 19 respondents to an open-ended question on the content of the recent SBC guideline revision answered correctly. Only 8 (42%) of these respondents stated that the change in guidelines influenced them to change their practice, but this may reflect a high proportion already ordering SM for women younger than 50 years. Virtually all EHC internists reported conducting SBC, although they differed in the age at which screening is initiated and the frequency. Diversity of practice may reflect the differences in the recommended guidelines. Further study is indicated to assess a) the determinants of physician decisions regarding choice of screening protocols, and b) whether self-report accurately represents actual clinical practice.

Comparison of Two Rapid Streptococcal Diagnostic Tests with Bacterial Culture in an Ambulatory Setting. N. Rambaran, H. Rambaran, K. Chennapragada, and V. M. K. Bhaskarabhatla. Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

We are evaluating the efficacy of the Abbott Test Pak^{tm} and Quick Vue^{tm} "rapid tests" compared with throat culture for the detection of Group A β-hemolytic *Streptococcus.* It is well established that patients with complaints of acute pharyngitis usually have either a bacterial or viral etiology. Because therapy is indicated for Group A β-hemolytic *Streptococcus,* rapid diagnosis is crucial to the initiation of therapy. The conventional method of performing a throat culture usually takes 48 hours for results to become available. This delay may result in less effective therapy especially with decreased compliance for follow-up in some patients. The availability of a rapid test which is sensitive and specific will not only enable therapy to be initiated more quickly for the benefit of the patient, but may also reduce the medical costs associated with this condition. In our study, patients presenting with clinical signs and symptoms of sore throat had both the Abbott Test Pak^{tm} and Quick Vue^{tm} tests as well as throat culture performed. Seventeen consecutive patients have been tested (average age, 9 years) to date. There was no difference in the sensitivity for the Quick Vue^{tm} compared with culture; each detected 4 (23%) of 17 cases. The Test Pack^{tm} detected 6 (35%) positive of 17 ($p = 0.71$, compared with culture data). Although only a few subjects have been studied thus far, preliminary results suggest that both the Abbott Test Pack^{tm} and Quick Vue^{tm} rapid tests may be used for the evaluation and management of patients.

Development of an Educational Program Targeted to Improve Patient Compliance with Medications: 1997. M. Rhee and R. A. David. Division of General Medicine, Mount Sinai School of Medicine, New York, NY.

Objectives: Compliance problems with medications are widespread. This is particularly true among Spanish-speaking patients (Rhee and David. J Gen Intern Med 1997; 12(suppl. 1):123). The language barrier that exists for these Spanish-speaking patients inhibits adequate education regarding their medications, and more importantly, has a negative impact on compliance. In response to these findings following goals were established: 1) to determine the most commonly prescribed medications; 2) to obtain and distribute patient educational materials concerning these medications written in both English and Spanish.

Methods: Through chart review, the data about the most commonly prescribed medications were obtained. This chart review consisted of 100 patients through consecutive sampling at Internal Medicine Associates (IMA), a multidisciplinary primary care ambulatory medicine site located in the Primary Care Building at Mount Sinai Medical Center. The data were collected for 1 week during consecutive patient visits to their primary care providers. Inquiries to major pharmaceutical companies and large drug store chains were made to acquire information about the existence of educational materials for patients.

Results: The most commonly prescribed medications were: albuterol (26 ± 8%), aspirin (23 ± 8%), lisinopril (20 ± 7%), acetaminophen (18 ± 7), nifedipine (14 ± 7%), furosemide (14 ± 7%), ranitidine (13 ± 7%), multivitamins (12 ± 6%), hydrochlorothiazide (11 ± 6%), nitroglycerin (10 ± 5%), NPH insulin (10 ± 5%), and calcium supplements (10 ± 5%), respectively (confidence intervals based on $p < 0.05$). Of additional interest, the most common diagnoses were: hypertension (55 ± 10%), diabetes mellitus (28 ± 9%), asthma (25 ± 8%), chronic ischemic heart disease (18 ± 7%), hypercholesterolemia (14 ± 7%), osteoarthritis (13 ± 7%), and depression (13 ± 7%) (confidence intervals based on $p < 0.05$). Of 15 major pharmaceutical companies surveyed regarding the above-mentioned medications, 60% did not offer patient educational materials, 20% provided them in English only, and 20% provided them in both English and Spanish. Two large drug store chains produced the materials only in English. The United States Pharmacoepia (USP) creates drug information leaflets available in both English and Spanish for all of these medications.

Conclusions: Because patients are less likely to take their medications without knowledge about side effects, we analyzed which drugs are most commonly taken and created an educational program to improve patient awareness and compliance. We will be distributing fact sheets on the most commonly used medications in both English and Spanish. We plan to monitor the results of our educational program.

This project was supported by the Patricia S. Levinson Summer Fellowship Program for medical students at Mount Sinai School of Medicine.

Pulmonary Embolus: New Principles for an Old Problem. G. Roshwalb, A. Dunn, and B. Chung. Mount Sinai School of Medicine, New York, NY.

Case: The patient is a 78-year-old man who presented to the emergency room complaining of 4 days of pedal edema and worsening dyspnea on exertion during the past 6 months. The patient noted that his exercise tolerance had gone from being able to walk many miles, to only being able to walk one block before having to stop and catch his breath. During the past 4 days, the patient noted an abrupt decline in his exercise tolerance, weight gain of about 10 pounds, and swelling in both his feet. The patient denied any chest pain, palpitations, sweating, fever, orthopnea, or weakness. The patient had a 15 pack-year smoking history, but stopped smoking in 1958, and was taking no medications. On physical examination, the patient was afebrile, had a pulse of 72, and blood pressure of 140/70. The neck examination showed minimal JVD, and the lung and heart examinations were normal except for an accentuated P2. The patient had bilateral pedal edema, and on rectal examination had a hard nodular prostate. Chest x-ray showed an enlarged heart, blunting of the right costophrenic angle, and a wedge-shaped density in the right lower lobe. VQ scan showed four perfusion defects, which was interpreted as a high probability for pulmonary embolism (PE). The diagnosis was chronic pulmonary emboli, and a prostate biopsy revealed adenocarcinoma.

Discussion: Physicians are taught early in their careers that eternal vigilance is necessary to diagnose PE when a patient presents with acute dyspnea or chest pain. In the patient presented above, however, the symptoms described occurred during a period of 6 months and were more compatible with a history of heart failure. Although eternal vigilance is useful in suspecting acute PE, what alerted the physicians in this case to the possibility of chronic PE was the discrepancy between the clinical history of left-sided heart failure and the physical findings of right-sided failure. We propose that along with a low index of suspicion, the necessity to reconcile the pathophysiology of physical findings that do not dovetail with the clinical history should be used to raise one's suspicion of PE. A second question raised by this case is how far to pursue a malignancy work-up in a patient presenting with PE. Recent literature has indicated that the answer lies in evidence-based medicine. Studies have shown that 10% to 23% of these patients have cancer or will manifest a malignancy soon after this diagnosis is made. Although a significant number of these patients will have cancer, authorities do not recommend an extensive work-up. A thorough history, physical examination, hemogram, liver function tests, chest x-ray, and the routine age- and gender-specific cancer screening tests should be done. In this case, a rectal examination and biopsy led to the diagnosis of adenocarcinoma of the prostate. This further illustrates that the evaluation for cancer should not be forgotten when working up patients with chronic PE.

Prevalence, Treatment, and Outcome of Obesity in Medical Primary Care at Elmhurst Hospital Center. M. Shah. Department of Medicine, Elmhurst Hospital Center, NY.

Significant morbidity and mortality are associated with obesity, one of the most common problems encountered in the primary care setting. A retrospective cohort study was conducted to ascertain the prevalence, treatment offered, and outcome of obesity in a primary care clinic serving a low-income, multiethnic population. A convenience sample of patient care sessions was chosen, and a systematic random sample was selected from appointment lists for the chosen sessions. Data extracted from the chart included demographic information, weight, height, documentation of diagnosis, components of management, and weight loss or gain after entry into the clinic. Ideal body weight and body-mass index (BMI) were calculated. Twenty-five patients were included in the sample. Of these, 52% were Hispanic and 28% were white, 64% were women. Obesity prevalence differed by age (52% of those 51 to 65 years old, 32% of those 36 to 50 years old, and only 12% of those more than 65 years old). Comorbid conditions were more prevalent in those with a BMI = 31–32 (32%) vs those patients with BMI = 36–40 (28%). The diagnosis of obesity was not addressed in 56% of the charts. Of those in whom obesity was diagnosed, 8% received advice to exercise, 36% received education on their condition, and 96% of patients received dietary instruction or a referral for consultation with a nutritionist. Patients with a BMI ⩾ 36 received more interventions using a multidisciplinary approach than those patients with a BMI of ⩽35. At the end of 2 years of follow-up, a weight loss of 6–10% occurred in only 8% of patients, and a weight gain of greater than 5% was found in a substantial proportion. In conclusion, a greater proportion of patients should receive management of their obesity, including education, exercise prescriptions, and referral to a nutritionist or a weight-loss group.

Identification of Patients with Occupational Risk Factors for Osteoarthritis of the Knee: 1997. A. Vora and S. Richman. Department of Community Medicine, Mount Sinai School of Medicine, New York, NY.

Osteoarthritis of the knee is the leading cause of chronic disability among the elderly in the United States. Known risk factors for osteoarthritis include age, joint trauma, repetitive occupational use causing chronic joint trauma, obesity, and genetic factors. Workers in occupations involving awkward posture and joint misalignment have a significantly increased risk of developing osteoarthritis, and up to 32% of knee osteoarthritis is attributable to occupational exposures. The prevalence of knee osteoarthritis related to occupational risk factors among patients with osteoarthritis in a general geriatric practice was examined in a pilot study. Finding a significant amount of occupationally related osteoarthritis would suggest the utility of interventions for modifiable risk factors. Patients with osteoarthritis in a general geriatric practice were interviewed using an open-ended questionnaire about occupational history, physical movements related to jobs, and other risk factors for arthritis. Patients were queried at the time of their scheduled appointments; there was 100% participation. Demographic data and body mass index were obtained from patient charts. Patients with more than 10 years of exposure to known occupational risk factors for knee osteoarthritis were categorized as having significant occupational exposure. Patients with dementia were excluded. Of 16 patients interviewed, 7 had significant exposure to known occupational risk factors for osteoarthritis of the knee. These included a 70-year-old retired factory worker with osteoarthritis after years of repetitive motion of the knee operating a press machine, with onset of knee pain at age 26 years. Two of the seven patients at high occupational risk had first-degree relatives with osteoarthritis, compared with three of the nine low-risk patients. Four of the seven patients at high occupational risk and seven of the nine at low risk were currently obese, using the most recent definition of obesity as body mass index greater than 27. The finding of this pilot study that 7 of 16 patients had significant occupational risk factors for osteoarthritis of the knee suggests the need for further investigation of these risk factors in a general geriatric practice. Given the high prevalence of osteoarthritis (60% over age 65 years), decreasing the burden caused by preventable, i.e., occupational causes, could greatly reduce the human suffering, disability, and high financial costs of knee osteoarthritis for the elderly population of the future.

Hematology

***†The HMG Domain Protein SSRP1 is an Architectural Transcription Factor Involved in Activation of the Human Embryonic β-like Globin Gene.** M. A. Dyer, P. J. Hayes, and M. H. Baron. Department of Molecular and Cellular Biology, Harvard University, Boston, MA; and Division of Hematology, Department of Medicine, Mount Sinai School of Medicine, New York, NY.

A fundamental requirement of development in multicellular organisms is the spatial and temporal regulation of specific subsets of genes. The vertebrate globin gene families have provided an important model for understanding the molecular basis for such differential gene expression. During development, distinct members of each of the globin gene families (α and β) are expressed sequentially in red blood cells (erythroid cells). The human embryonic β-like globin gene (ε) is expressed in primitive erythroid cells of the yolk sac during the first few weeks of gestation. We have previously shown that developmental stage-specific expression of the ε-globin gene is mediated by multiple positive and negative regulatory elements upstream of the start of transcription. Of particular interest is one positive regulatory element, PREII, that works together with an upstream element, PREV, to confer tissue- and developmental stage-specific expression on a minimal promoter (Mol Cell Biol 13:7457–7468). We have identified an ~85 kDa PREII binding factor (PREIIBF) that is enriched in erythroid cells. This protein has been characterized biochemically and shown to bind specifically to a novel 19-bp region within PREII (Mol Cell Biol 14:3763–3771). PREIIBF bends the target DNA at PREII and may therefore mediate synergistic interactions with other positive regulatory elements by looping out of the intervening DNA (Mol Cell Biol 16:829–838). Here we present the cDNA expression cloning of PREIIBF. The cDNA encodes a previously identified member of the HMG-domain protein family termed SSRP1. Interestingly, a hallmark of human menopausal gonadotropin (HMG)-domain proteins is their ability to bend DNA. The size of PREIIBF purified from human erythroleukemia cells is identical with that of the predicted translation product of SSRP1. The DNA binding specificity of recombinant SSRP1 expressed in bacteria or insect cells is identical with that of PREIIBF. PREIIBF purified from erythroid nuclear extracts is recognized by antisera raised against synthetic peptides of SSRP1. Therefore, by several biochemical and immunological criteria, we conclude that PREIIBF is encoded by the SSRP1 gene. Other HMG proteins have been shown to regulate gene expression by coordinating the assembly of multiprotein complexes. Proteins bound at multiple sites within the upstream regulatory region of the human ε-globin gene may form a stereospecific multiprotein complex to activate ε-globin transcription in embryonic erythroid cells.

†Dominantly Inherited Chorea-Parkinsonism-Dementia-Acanthocytosis. R. H. Walker,[1] A. Melnick,[2] B. Davidoff-Feldman,[3] and M. F. Brin.[1] [1]Department of Neurology and [2]Division of Hematology, Mount Sinai School of Medicine, New York, NY; and [3]Nassau County Medical Center, NY.

Acanthocytes are abnormally shaped red blood cells which have a characteristic spiculated appearance and often have shortened survival times. Acanthocytes are associated with three movement disorders, abetalipoproteinemia (or hypobetalipoproteinemia), Mcleod phenotype, and neuroacanthocytosis (NA). NA is a heterogeneous disorder for which the inheritance has not been established. We have described a family with a novel form of NA with apparent autosomal dominant inheritance. A 54-year-old man was referred for evaluation of a movement disorder with severe dementia. The patient's mother and maternal grandmother had "jerky movements" and cognitive decline. The patient has a 20-year history of cognitive decline and choreiform movements; he had been bed-bound for 5 years. A peripheral blood smear showed acanthocytes. Analyses of red cell Kell and Kx antigens were negative for Mcleod phenotype. Creatine kinase and apolipoprotein B were normal. Genetic testing for Huntington's disease and dentatopallidorubroluysian atrophy was negative. Magnetic resonance imaging scans of the brain showed severe atrophy of the basal ganglia, particularly the caudate and cortex. The results of electromyograms and nerve conduction velocity studies were normal. Nerve biopsy was nondiagnostic, and muscle biopsy showed nonspecific diffuse atrophy. Scanning electron microscopy was performed and showed increased proportions of acanthocytic red cells as compared with a normal control. Sodium dodecyl sulfate-gel electrophoresis showed an abnormal pattern of red cell membrane proteins. The observed novel band is most likely consistent with an abnormal band 3 component. The patient's 32-year-old nephew (son of his mother's sister) has a 3-year history of cognitive decline and mild parkinsonism. Peripheral blood smear showed acanthocytes. We report here a family with a novel hereditary neurodegenerative disorder with acanthocytosis, which is inherited by either autosomal dominant (most-likely) or X-linked genetics and has unique biological characteristics. This family does not correspond to Mcleod or abetalipoproteinemia phenotypes. The pathophysiology of this disorder may be linked to abnormal band 3 proteins, a topic which is being investigated further.

The Cure of Acute Myelocytic Leukemia in Adults Younger than 60 Years of Age. R. Afonja, G. H. Rubin, D. M. Todd, and A. D. Rabin. Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

Prolonged disease-free survival (DFS) in patients younger than 60 years of age with acute myelocytic leukemia (AML) using conventional chemotherapy is possible in only 20% to 30% of cases. Allogeneic bone marrow transplant yields 40–60% DFS, but as much as 25% succumb to regimen-related toxicity. Furthermore, a suitable allogeneic donor can be found in only a few of these patients. Successful autologous bone marrow transplantation has been plagued by prolonged pancytopenia and frequent relapse, therefore existing therapeutic regimens available for treating these AML patients are risky and offer limited success. We now report a new regimen using autologous peripheral stem cells isolated after an *in vivo* purge, yielding superior results with minimal risk. Thus 25 patients younger than 60 years of age with untreated AML (M1–M6) with both favorable and unfavorable subtypes were induced into CR-1 by high-dose cytosine arabinoside (HIDAC) and high-dose daunorubicin. After 1 month in CR-1, an *in vivo* purge was achieved by HIDAC and VP-16. Peripheral stem cells were isolated after granulocyte colony-stimulating factor stimulation. In a third chemotherapy course, patients were given myeloablative doses of busulfan and VP-16, and stem cells were reinfused. There were no regimen-related deaths or life-threatening toxicity. Overall, actuarial survival was 85% and DFS was 65% during a 4.5-year interval. Of 19 patients brought through to transplant, there has only been one relapse. No patient with a favorable AML subtype, t(15;17), t(8;21) and inv(16), has had a relapse so far. If time and if additional patients bear out these preliminary results, we can offer what appears to be the safest and most successful treatment for AML yet reported. Some patients are part of a study conducted in collaboration with Dr. C. Linker, UCSF.

A Study of Cyclosporin A and Imipenem Grand Mal Seizure in Allogeneic Bone Marrow. S. Ahmad, N. S. Turhal, I. Isola, E. Scigliano, A. Vlachos, and S. Fruchtman. Division of Hematology and Bone Transplant, Mount Sinai School of Medicine, New York, NY.

Imipenem (I) is a β-lactam antibiotic used with Cilastatin© in the bone marrow transplant (BMT) setting for the treatment of infections caused by aerobic and anaerobic organisms. The predominant concern regarding its use is the tendency to induce seizures, which is associated with inadequate dose adjustments in relation to renal function. Cyclosporin A (CsA) is a fungal product used in the BMT setting as an immunosuppressive agent with no myelotoxicity. It is associated with nephrotoxicity and hypertension. Seizures can be seen with CsA therapy, in both solid organ and allogeneic BMT recipients. Our hypothesis for study was that central nervous system toxicity and seizures would be increased by the concomitant administration of both CsA and I/C.

Methods: Between December 1989 and December 1996, 166 of the stem cell BMTs performed at Mount Sinai Hospital were either allogeneic ($n = 122$) or autologous ($n = 44$) and evaluated for this study. All allogeneic patients received CsA 1.5 mg/kg b.i.d. as part of their immunosuppressive regimen, starting 1 day before infusion of stem cells. Sixteen patients received prophylactic Dilantin when Busulfan was used as part of the preparative regimen. Thus, three groups were studied: 77 patients received CsA alone without I/C (group 1); 89 patients received I/C, of these, 45 received concomitant CsA (group 2); 44 patients who underwent autologous BMT received I/C only and no CsA (group 3).

Statistical Analysis: Differences between proportions were evaluated using a two-tailed Fisher's exact test (as well as the uncorrected test for differences in proportions). A t-test was used to compare baseline values of the patients with and without seizures with respect to blood chemistries. Wilcoxon's nonparametric test was used if the data were markedly nonnormal, typically becuase of skewness or extreme outliers. These two-sample tests were also used to compare the values of the chemistries at seizure with baseline values for patients without seizures. Changes between baseline and chemistries at the time of seizure were examined for five patients who had seizures.

Results: We observed 3 (3.8%) seizure episodes in patients who were receiving CsA alone (group 1). There were also 2 (4.4%) seizure episodes in patients who were receiving I/C and CsA (group 2). Group 3 had no seizures. There were no statistical differences between groups 1 or 2. We studied electrolytes, liver and renal function influences under which patients will have seizures; the only statistically significant difference found was hyperbilirubinemia. Adverse effects may be difficult to relate to a particular drug, especially for patients receiving multidrug regimens. There was no history of seizures, and baseline multiple blood chemistries and functional parameters were not statistically different in the two groups. The number of days patients were receiving CsA and I/C did not differ significantly from patients who received both and did not seize.

Conclusion: The use of I/C and CsA did not cause a significant rise in the frequency of seizures compared with CsA alone. An antibiotic regimen including I/C should be considered for neutropenic febrile allogeneic BMT patients in whom first-line antibiotic therapy fails. It appears that this regimen does not increase the risk of seizures in this patient population receiving CsA.

The Human Very Low Density Lipoprotein Receptor Mediates the Uptake and Degradation of Lipoprotein Lp(a). K. M. Argraves, J. T. Fallon, D. K. Strickland, and P. C. Harpel. Mount Sinai School of Medicine, New York, NY; and the Holland Laboratory, American Red Cross, Rockville, MD.

Lp(a) is an important risk factor for premature atherosclerosis. The mechanisms for Lp(a) atherogenicity most likely involve both its capacity to influence plasminogen activation as well as its atherogenic potential as a lipoprotein particle after receptor-mediated uptake. The mechanisms responsible for the cellular catabolism of Lp(a) are currently a topic of debate. We demonstrate that fibroblasts expressing the human very low density lipoprotein (VLDL) receptor can mediate the endocytosis of Lp(a) leading to its degradation within lysosomes. In contrast, fibroblasts deficient in this receptor are not effective in catabolizing Lp(a). Lp(a) degradation was prevented by antibodies against the VLDL receptor, and by RAP, an antagonist of ligand binding. The catabolism of Lp(a) was also inhibited by apolipoprotein(a), indicating that apo(a) mediates the binding of Lp(a) to this receptor. These data indicate that the VLDL receptor is capable of binding and mediating the catabolism of Lp(a). We also demonstrate the expression of the VLDL receptor in macrophages present in human atherosclerotic lesions. The ability of the VLDL receptor to mediate endocytosis of Lp(a) could lead to cellular accumulation of lipid within macrophages, and may represent a molecular basis for the atherogenic effects of Lp(a).

Regulation of Embryonic Hematopoiesis in Yolk Sac Mesoderm by Endodermal Signaling Molecules. M. Belaoussoff, S. M. Farrington, and M. H. Baron. Department of Molecular and Cellular Biology, Harvard University, Boston, MA; and Division of Hematology, Department of Medicine, Mount Sinai School of Medicine, New York, NY.

The determination of cell fate during embryogenesis is governed by instructive and permissive signals emitted and received between different populations of cells in a phenomenon called embryonic induction. In nearly all vertebrate animals, embryonic blood development begins during gastrulation and results from the induction of extraembryonic (ventral) mesoderm to form hematopoietic tissue. Previous studies on developing chick embryos suggested that signals from extraembryonic endoderm were required for primitive hematopoiesis in extraembryonic mesoderm, but the nature of these signals was unknown. We have devised a novel transgenic mouse embryo explant culture system to determine whether extraembryonic (visceral) endoderm signals are required for induction of mammalian embryonic blood formation. In this assay, embryos carrying a human embryonic β-like globin regulatory region linked to a lacZ reporter gene serve as a source of genetically marked cells in which blood formation can be followed by histochemical staining for lacZ activity. Embryos are harvested before the onset of gastrulation (i.e., at a time before blood development begins) and are cultured for 2 to 4 days. They are then stained for lacZ expression or are harvested individually for analysis by semiquantitative (multiplex) reverse transcriptase-polymerase chain reaction. We have shown, using a tissue recombination approach, that blood formation in the mouse embryo is not autonomous to hematopoietic mesoderm alone: that is, the process requires signals from the closely apposed layer of visceral endoderm cells. Furthermore, we have shown, using conditioned medium from a visceral endoderm cell line, that the signals are diffusible and that direct cell contact between extraembryonic endoderm and mesoderm is not required. These signals may be required for embryonic vasculogenesis as well as hematopoiesis. Our studies demonstrate that visceral endoderm signaling to hematopoietic mesoderm is conserved between mouse and chick. They also establish a biological assay for signaling molecules involved in early hematopoiesis and vasculogenesis.

Two Unrelated Patients with Glanzmann Thrombasthenia Have an Identical Mutation Resulting in a Deletion of the Transmembrane Domain in Platelet GPIIb. D. L. French, F.-P. Chen, L. Xu, J. Weiss, and B. S. Coller. Mount Sinai School of Medicine, New York, NY and St. Luke's Roosevelt Hospital, New York, NY.

Three new mutations have been identified in two patients (CG and MC) with Glanzmann thrombasthenia. Both patients had typical clinical and laboratory findings. Platelet surface GPIIb/IIIa receptors were expressed at levels of 5% to 11% of normal, and $\alpha_v\beta_3$ receptors were expressed at levels of ~200% of normal, which suggests defects in GPIIb. Immunoblot analyses of whole platelet lysates showed reduced levels of GPIIb and GPIIIa, but normal proteolytic processing of GPIIb. The platelet fibrinogen level of patient CG was ~20% of normal and of patient MC was ~12% of normal. Direct sequence analysis of polymerase chain reaction (PCR)-amplified fragments identified the patients as compound heterozygotes. Both patients have an identical T-to-C mutation in the splice donor sequence of GPIIb exon 29 resulting in a predicted inframe deletion of the transmembrane domain. This deletion was confirmed by reverse transcriptase PCR amplification of patient RNA and sequencing of cloned fragments. The other mutation in patient CG results in a GPIIb Ile565Thr substitution and the other mutation in patient MC results in a TG base pair insertion in the cytoplasmic domain of GPIIb. This insertion causes a frame shift, but no premature termination resulting in reduced levels of RNA. The first 11 residues of the cytoplasmic domain, including the conserved GFFKR motif, were unchanged by the mutation, whereas the remaining residues, including the acidic rich region, were all altered. We infer that each of these new mutations affects expression of GPIIb/IIIa and may provide important information on GPIIb/IIIa structure, function, and biosynthesis.

Patients with Dysfibrinogenemia Have a High Incidence of Hepatitis C Virus Infection. I. Genkin, L. Feinman, and T. Muhlfelder. Hematology/Oncology Section, Mount Sinai School of Medicine, New York, NY; and Bronx VA Medical Center, NY.

Acquired dysfibrinogenemia occurs mainly in patients with liver disease. The abnormality believed to be primarily responsible for acquired dysfibrinogenemia in liver disease is secondary to hyperglycosylation of the fibrinogen molecule with sialic acid molecules. A diagnosis of dysfibrinogenemia requires a prolonged thrombin clotting time, a normal fibrinogen concentration, and a normal or minimally elevated concentration of fibrin/fibrinogen degradation products (FDP). We reviewed the results of patients who had full coagulation studies in the Special Hematology laboratory at the Bronx VA Medical Center since 1992. We identified 39 patients who had dysfibrinogenemia and who had been tested for hepatitis C virus (HCV). Of these 39 patients, 27 were HCV+ and 12 were HCV–, for a 69% positivity rate. In contrast, of 87 patients without dysfibrinogenemia, 35 were HCV+ and 52 HCV–, for a 40% positivity rate ($p < 0.05$). Serologic evidence for hepatitis B was low and approximately equal in both groups. Patients who were HCV+, without dysfibrinogenemia, had significantly higher albumin levels than HCV+ patients with dysfibrinogenemia. However, they did not differ significantly in their prothrombin time levels or bilirubin levels. There were no significant differences in the three parameters when HCV+ and HCV– patients with dysfibrinogenemia were compared. As far as we know, this is the first reported association of dysfibrinogenemia with HCV infection. It is yet unclear whether HCV infection causes dysfibrinogenemia independent of its effect on liver function impairment, because only albumin concentrations were significantly lower in HCV+ patients with dysfibrinogenemia. Studies are underway to answer these questions.

Haplomismatched Allogeneic Bone Marrow Transplant using CD34$^+$ Selected Bone Marrow Cells. L. Isola, D. Skerrett, J. Lipton, A. Vlachos, V. Najfeld, E. Scigliano, and S. Fruchtman. Divisions of Hematology and Pediatric Hematology/Oncology, Mount Sinai School of Medicine, New York, NY.

Lack of a HLA-matched donor excludes two thirds of patients who could benefit from allogeneic bone marrow transplant (BMT). The use of haplomismatched donors (e.g., siblings and parents) greatly expands the scope of BMT. Two elements have been defined as crucial to the success of haplomismatched allogeneic BMT, a large stem cell dose and a high degree of T-cell depletion. As yet, no T-cell depletion technique has received FDA approval. CD34$^+$ selection is FDA approved for autologous BMT and can achieve both goals. However, in adults, BM CD34$^+$ selection yields a low stem cell dose. In children, theoretical calculations indicated that BM CD34$^+$ selection may provide an adequate stem cell and T-cell dose for haplomismatched transplantation. A 4-year-old boy with juvenile chronic myelogenous leukemia (JCML) underwent a haplomismatched allogeneic BMT at Mount Sinai Medical Center using CD34$^+$-selected cells from his father. The preparative regimen included cyclophosphamide, total body irradiation, and ATG. The post-transplant support and graft-vs-host disease (GVHD) prophylaxis used were standard. The patient's father was a one haplotype mismatch. He underwent a BM harvest of 1,680 mL. Cell processing was accomplished using a Ceprate SC instrument (CellPro, Bothell, WA) under an IRB approved protocol that involves an FDA IDE. Details of the cell processing follow.

	Total nucleated count	CD34 %	CD34 content	CD3 %	CD3 content	Viability %
Harvest	3.1×10^{10}	1.1	3.4×10^{8}	68	2.1×10^{10}	100
Postcolumn	1.5×10^{8}	31.0	0.5×10^{8}	25	0.4×10^{8}	96

The total CD34$^+$ dose adjusted to body weight was 3×10^6/kg and the CD3$^+$ cell dose 2.2×10^6/kg. The fold T-cell depletion was 560 (2.5 logs) and the CD34$^+$ yield was 15%. The patient achieved trilineage engraftment 1 month post-BMT but unfortunately relapsed soon after. Moderate skin and gut GVHD was seen 2 weeks postengraftment and resolved with standard dose corticosteroids. BM CD3$^+$ selection provides a stem cell product that may be used for pediatric haplomismatched transplantation. Its application to adults is now limited by stem cell dosing. *In vitro* expansion may allow extending this technology to all HLA-mismatched recipients.

Induction of Basic Helix-Loop-Helix Protein-Containing Complexes During Erythroid Differentiation. J. A. Lister and M. H. Baron. Department of Molecular and Cellular Biology, Harvard University, Boston, MA; and Department of Medicine, Mount Sinai School of Medicine, New York, NY.

Commitment and differentiation of hematopoietic stem cells along the erythroid lineage is controlled in part by tissue-restricted transcription factors. These include proteins of the basic helix-loop-helix (bHLH) family, which derives its name from a conserved structural motif consisting of a short basic region required for DNA binding, followed by two amphipathic α-helices separated by an intervening loop of variable length. The HLH domain mediates dimerization, and dimers of bHLH proteins recognize a consensus element which is found in the promoters and enhancers of many cell-specific genes and is known as the E-box. The involvement of bHLH transcription factors in erythroid differentiation and development has been established by forced expression of the proteins TAL1 and Id1 in cultured cell lines and by targeted disruption of the mouse *TAL1* gene. To better understand the mechanism by which bHLH proteins regulate erythropoiesis, we have investigated HLH protein-DNA interactions in mouse erythroleukemia (MEL) cells before and during chemically induced differentiation. Thee bHLH (E-box) binding activities were found to be induced in nuclei from differentiating MEL cells. Using specific antisera, we demonstrated that these complexes are dimers of TAL1 and ubiquitous E proteins. Similar complexes were detected in nuclear extracts from a human erythroid cell line, K562, and from mouse fetal liver. All three bHLH complexes were disrupted *in vitro* by Id1, a dominant-negative HLH protein that we and others have previously shown to antagonize MEL cell differentiation. During differentiation of an Id1 overexpressing MEL cell line, induction of a complex containing TAL1 and E2A was not only blocked but reduced below the levels seen in undifferentiating cells. These observations are consistent with the idea that TAL1 and Id1 have opposing effects on erythroid differentiation and that the level of TAL1/E2A heterodimer and/or another E-protein-containing complex may influence the decision of a cell to terminally differentiate.

Novel Variant Translocations Occurring in Acute Myelogenous Leukemia. A. Melnick,[1] S. Fruchtman,[1] A. Zelent,[2] B. Boczkowska,[1] M. Liu,[3] Q. Huang,[3] M. J. Calasanz,[4] A. Fernandez,[4] J. D. Licht,[1] and V. Najfeld.[1] [1]Division of Hematology, Mount Sinai School of Medicine, New York, NY; [2]Institute of Cancer Research, UK; [3]University of Navarra and Hospital, San Milagros, Spain; and [4]Shanghai Institute of Hematology, China.

Chromosomal translocations have been found to be associated with multiple different human malignancies. The study of the resulting abnormal gene products has led to fundamental advances in the understanding of cancer biology. This study describes the first time the t(2;15)(p23;q22) and t(2;17)(p23;q21) translocations have been identified in human malignancy. All loci involved in these translocations include genes that are rearranged in other cancers: 1) The retinoic acid receptor alpha (RARα) gene is a nuclear hormone receptor rearranged in all cases of acute promyelocytic leukemia (FAB M3-type AML). 2) The promyelocytic leukemia (PML) gene on 15q22, encoding RING-finger nuclear protein of unknown function, is involved in most cases of M3 AML as well. 3) The anaplastic lymphoma kinase (ALK) gene on 2p23 is a tyrosine kinase receptor involved in many anaplastic large cell lymphomas. Patient 1, a 73-year-old man in whom myeloblastic (FAB M1 sub-type) AML was diagnosed, was treated with standard chemotherapy. Six months later he relapsed and was referred to this hospital. He was pancytopenic and had a hypercellular bone marrow with 20% myeloblasts. Cytogenetic analysis in all 30 metaphases studied showed a 47,XY,t(2;15)(p23;q22),+13 karyotype. Fluorescent *in situ* hybridization (FISH) was done to determine whether the PML gene on chromosome 15q22 was rearranged. PML was transferred intact to the short arm of chromosome 2 without any breakage. FISH of the ALK gene locus on chromosome 2p23 showed likewise, that this gene was transferred passively to the long arm of chromosome 15 without any translocation. Patient 2 was a 60-year-old man who presented with fatigue, weight loss, and dyspnea. He had a granulocyte count of

150,000 mm^3, thrombocytopenia and macrocytic anemia, and generalized lymphadenopathy. Bone marrow morphology and cytochemistries were consistent with monocytic (FAB M4-type) AML. Cytogenetic analysis was performed and all 50 metaphases analyzed showed 46,XY,t(2;17)(p23;q21) karyotype. FISH was performed to detect possible rearrangement of RARα known to be located on chromosome 17q21. RARα was passively transported to the short arm of chromosome 2 but was not translocated. In conclusion, none of the coding regions of the three genes studied were translocated in these patients. This raises the possibilities that 1) other neighboring genes could be involved in these translocations and 2) that noncoding regulatory sequences of the studied genes could be put in contact with and deregulate expression of other genes. Alternatively, displacement of ALK, RARα, and PML to novel positions could lead to loss of their normal regulation (position effect).

Successful Management of Hepatic Veno-occlusive Disease by Antithrombin-III After Stabilization of the Disease by Combined Plasma Exchange and Tissue Plasminogen Activator. H. Mohtaseb, M. Lamacchia, G. Rubin, G. Hajjar, D. Todd, S. Stives, and A. D. Rubin. Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

Veno-occlusive disease (VOD) of the liver, a clinical syndrome characterized by fluid retention, hepatomegaly, and hyperbilirubinemia, is common and increasingly recognized as an early complication of high-dose chemotherapy, occurring primarily after allogeneic bone marrow transplantation (BMT). Treatment is mainly supportive because there is no effective therapy so far that reverses the microvascular occlusive process of intrahepatic venules with its associated centrilobular hepatocyte and sinusoidal damage. We are reporting a successful response of severe VOD to antithrombin-III and plasmapheresis. A 32-year-old woman with a diagnosis of chronic myelogenous leukemia (CML) was treated with umbilical cord blood (UCB) transplantation in the chronic phase from a partially HLA-matched donor. The conditioning regimen included hyperfractionated total body irradiation during 4 days followed by Melphalan 60 mg/m^2 and ATG 30 mg/kg for 3 days. Graft-vs-host disease (GVHD) prophylaxis included methotrexate and cyclosporin. The patient received low-dose heparin infusion for VOD prophylaxis. On day +12 the patient developed full-blown, progressive VOD with a weight gain of 11.5 pounds, ascites, tender hepatomegaly, bilirubin rising to 15 mg/dL, and changed mental status. Plasma exchange was initiated with FFP replacement and tissue plasminogen activator at a dose of 20 mg of infusion for 3 days every week × 3 with continuous heparin infusion, which resulted in transient stabilization of the clinical condition and of the hyperbilirubinemia. On day +38 the patient received antithrombin-III infusion, 50 μ/kg every 8 hours × 3 followed by 80 mg/kg/day for 4 days. This resulted in dramatic and sustained resolution in the clinical and biochemical parameters of VOD. However, despite effective management of this life-threatening complication, the patient did not engraft even after repeated UCB infusion; she died on day +107 of disseminated aspergillosis. Throughout treatment, the VOD continued to be under control with near resolution of all its criteria. We conclude that antithrombin-III may be an effective new modality in treating one of the most common causes of death caused by cytotoxic therapy in BMT.

Remission Reinduction by Inducing Graft Versus Leukemia Effect in Acute Myelocytic Leukemia Relapsed after Allogeneic Bone Marrow Transplantation. H. Mohtaseb, G. H. Rubin, D. M. Todd, and A. D. Rubin. Department of Hematology and Oncology, St. Joseph's Hospital and Medical Center, Paterson, NJ.

Relapse of acute myelocytic leukemia (AML) after successful bone marrow transplantation (BMT) previously has left little hope for prolonged survival. Reinduction by chemotherapy or a second transplant is nearly always futile. We have been using donor leukocyte infusions (DLI) plus interleukin 2 (IL-2) to re-establish the donor graft for a stable complete remission (CR). Two women in full hematologic relapse of AML <1 year after successful BMT in CR2 from an HLA-matched brother were each given a DLI, which had been isolated by apheresis after granulocyte colony-stimulating factor stimulation. Recipients were then started on daily subcutaneous administration of IL-2. CR was promptly achieved in one patient. Donor marrow cells, identified by fluorescence *in situ* hybridization (FISH) and restriction fragment length polymorphism (RFLP) analysis, increased from 50% to 100%. In one patient, extramedullary evidence of disease persisted but remained controlled by IL-2. The second patient required an additional boost of chemotherapy before achieving a CR with 100% engraftment of donor cells. In neither case was there evidence of significant graft versus host disease (GVHD). In a third case, a 22-year-old woman with primary refractory AML 47XX(+8) successfully received a transplant of marrow from a partially matched male cousin. She had developed transient cutaneous acute GVHD. At 6 months, she relapsed with 70% leukemia cells by FISH. Cessation of cyclosporin resulted in GVHD in the form of BOOP treated successfully with corticosteroids. However, her marrow gradually improved exhibiting full engraftment in 3 months. She is clinically well with normal peripheral counts on tapering doses of corticosteroids. It is likely that in these cases the immunologic graft versus leukemia effect played a predominant role in driving off the recrudescent leukemia blasts and in reconstituting the marrow with donor hemopoietic elements. We conclude that AML patients, relapsed after BMT, can be brought successfully back into stable engraftment and hematologic remission without the risk of a second transplant. This offers a new opportunity for using adoptive immunotherapy in the management of AML.

Dendritic Cell Generation from Human Bone Marrow and Peripheral Blood $CD34^+$ Stem Cells *in Vitro*. L. A. Mueller, R. S. Weinberg, T. Sukkarieh, M. Cohen, L. Miller, C. Kiang, and L. M. Isola. Divisions of Pediatric Hematology/Oncology, Hematology, and Center for Clinical Labs, Mount Sinai School of Medicine, New York, NY.

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that activate immunologically naive T cells. The ability to generate DCs in sizeable numbers has significant implications in the immunotherapy of cancer. DCs generated *in vitro* have been used in lymphomas, melanomas, and prostate cancer with tumor regression. We are contemplating the use of DCs to improve immune antitumor response in patients with leukemia, lymphomas, and breast cancer after bone marrow transplantation (BMT). We established an efficient system for the generation of DCs in culture. Granulocyte colony-stimulating factor (G-CSF)-primed peripheral blood (PB) and bone marrow (BM) or non-primed BM were used as sources of stem cells. CD34+ cells were selected from PB or BM with a magnetic cell separation system (MACS; Miltenyi Biotech Inc., Auburn, CA). Cells were then cultured in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% fetal bovine serum (FBS), 100 ng/mL human recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF), 50 ng/mL stem cell factor (SCF), 50 ng/mL interleukin-4 (IL-4), and either with or without tumor necrosis factor-α (TNF-α). The cells were cultured in six-well tissue culture plates at a density of 1.5×10^6 cells/cc of medium. The cells were demidepleted every week when the cells became confluent and counted in duplicate. Cells were analyzed for dendritic morphology using light microscopy, as well as by fluorescence-activated cell sorter analysis using the T6-RD1 CD 1a antigen, and immunohistochemistry using an immunoperoxidase stain with a monoclonal CD1a antibody. DC cultures were maintained for at least 4 weeks to evaluate DC production and amplification from $CD34^+$ progenitors. Our results show that both by morphology as well as by CD1a analysis there is at least a 24-fold increase in the number of dendritic cells derived from CD34+ stem cells. For a typical input of 3×10^5 $CD34^+$ cells, the cumulative output of DCs after 4 weeks was greater than 2×10^6 cells. Over time, as the pool of CD34+ cells diminish, the numbers of DC generated reaches a steady state (approximately 2–3 weeks). The numbers of DCs generated demonstrates moderate expansion of cells that have DC morphology and bear the CD1a marker on the cell surface. The total cell yield is within the range of cells needed in the transplant setting. This number is analogous to those used in T-cell infusions after allogeneic BMT.

Hereditary Hemochromatosis Haplotype Defined by Histocompatibility and Microsatellite Alleles. D. Skerrett, O. Rosina, E. El-Harazy, O. Gudzowaty, and P. Berk. Divisions of Hematology and Liver Disease, Mount Sinai School of Medicine, New York, NY.

The hemochromatosis gene (HG) is located near the HLA A locus on chromosome 6. HLA antigens A3, A11, B7, and B14 and chromosome 6 microsatellite alleles, D6S265-1, D6S128-2, and D6S105-8, are in linkage disequilibrium with the HG and can be used as markers for disease association. The relative risk of disease association of HLA A3.2 and D6S105-8 is 9.1 and 48, respectively. We used molecular analysis of HLA and microsatellite alleles for the identification of the hemochromatosis haplotype in a family study. The family contained two siblings with hemochromatosis and two siblings with suspected abnormal iron metabolism. Analysis of HLA antigens, isotypes, and alleles was performed by standard lymphocytotoxicity, isoelectric focusing, and per-sequence specific oligopeptide probes, respectively. Microsatellite analysis of dinucleotide repeats at the D6S105 and D6S265 loci was performed on amplified genomic DNA using allele-specific primers and polyacrylamide gel electrophoresis. The affected individuals shared the haplotype, A3.2, D6S105-8, D6S265-1, an established haplotype with a 95% association with the hemochromatosis HLA H mutation, Cys282Thr. The other siblings carried the D6S105-8 allele without A3 or D6S265-1. HLA A1101, an allele with serologic cross-reactivity with A3, was present in all siblings but was not associated with the hemochromatosis haplotype. HLA 3.2 was identified as the marker for the hemochromatosis haplotype in this family. Subsequent screening in this family can be performed with serological screening for HLA A3. HLA typing is a rapid, informative, and cost-effective method of screening for disease in a family with identified haplotypes. This case documents the combined use of classical and nonclassical histocompatibility allele typing in defining haplotypes for disease association.

Comparative Molecular Analysis of HLA-B*8101. D. Skerrett, O. Rosina, E. El-Harazy, L. Isola, S. Fruchtman, G. Ross, E. Scigliano, and O. Gudzowaty. Division of Hematology, Mount Sinai School of Medicine, New York, NY.

A bone marrow donor evaluation was performed on a 24-year-old patient from Honduras, Central America. Standard lymphocytotoxicity revealed the patient's phenotype as A29, A33, ?B7/8101/48, B44 (12), Cw6, DR7, DR9, DQ2. Serologic reactivity with B7, 8101, and 48 was consistent with the B7 variant, B*8101. B*8101 is a known rare allele which has been described in an individual from Equatorial Guinea and shares a serological profile with B'DT'. Family studies confirmed the patient haplotypes: A 29, B44, DR7, DQ2 and A33, B8101?, DR9, DQ2. The B7 variant was not present in the available family members. One-dimensional isoelectric focusing identified isotypes: A29.2, A33.2, B44.1, and B7.1. Isotype B7.1 is a low-frequency (0.9%) isotype and has been associated with the B7 variant, B*0705. Isotype B7.1 has not previously been associated with B*8101. To further classify this allele, sequence-specific primer analysis of amplified, genomic DNA was performed. This technique identified a unique 206-bp nucleotide sequence confirming the B*8101 allele. The described PCR-SSP variant B*8101, shares sequence motifs with B*07, B*40, and B*4801. Nucleotide substitutions distinguish B*8101 from B*0705 at exon 3 (residues 158 and 161), exon 4 (residue 188), exon 5 (residues 21 and 119), and exons 1 and 2. The resulting amino acid substitutions are at the signal peptide, α_2 (143 and 147) and α_3 (245) domains, and transmembrane proteins. The α_3 Thr-245 substitution, also present in B*4801, many confer reduced CD8 affinity. The B*8101 proteins migrate to the isoelectric point (B7.1) shared with B7 variant, B*0705. Isoelectric focusing distinguishes the B7 variants and remains a useful technique for the identification of rare and novel class I alleles.

Ticlopidine-induced Thrombotic Thrombocytopenic Purpura. R. Theerthakarai, C. Balasingham, M. Kelly, A. D. Rubin, and D. Hanley. Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

The development of thrombotic thrombocytopenic purpura (TTP) secondary to treatment with ticlopidine is not widely recognized. This vignette reports two cases of TTP after the use of this agent. Case 1 was a 54-year-old white male patient with a history of inferior wall myocardial infarction (MI) and status: post stent to left anterior descending (LAD) coronary artery. The patient was started on ticlopidine (250 mg b.i.d.) and presented 25 days later with anorexia, nausea, left upper extremity numbness, severe thrombocytopenia, and microangiopathic hemolytic anemia (MHA). Ticlopidine was discontinued, and the patient underwent a 4-day course of plasmapheresis with reversal of symptoms. Case 2 was a 61-year-old white female patient with a LAD with stent who was started on ticlopidine (250 mg b.i.d.). Twenty-three days later, she presented with complaints of nausea and abdominal discomfort and was found to have severe thrombocytopenia, elevated lactate dehydrogenase, MHA, and renal insufficiency. The patient died of massive anterior wall MI before plasmapheresis could be initiated. Reports of possible ticlopidine-induced TTP all have come from outside the United States. Although we cannot establish a cause-and-effect relationship, we suggest that patients receiving ticlopidine should be monitored carefully for the development of TTP.

Infectious Diseases

"Giant" Blastoconidia of *Candida albicans*: Induction on Chocolate Agar and Morphologic Spectrum. J. Abrams, M. Horga, M. Cheng, S. Richards, and E. J. Bottone. Division of Infectious Diseases and Departments of Pediatrics, Mount Sinai School of Medicine. New York, NY.

Candida albicans, a yeast-like organism, produces oval budding cells called blastoconidia, measuring 2 to 4 μm. "Giant" forms (≈30 μm) of *C. albicans* blastoconidia were noted in a throat culture plated on to chocolate agar. Such giant blastoconidia have only been described when *C. albicans* is grown on a medium with xylitol. Giant blastoconidia were observed solely after growth on chocolate agar,, but not on blood and Sabouraud's agar. Giant blastoconidia ranged from 24 to 26 μm up to 30 μm; approximately 10 to 15 times the size of normal blastoconidia. Morphologic forms of giant blastoconidia included buds with a broad base and elliptically forms similar to oocysts of the *Isopora* species. Some produced giant pseudohyphae as well. Giant forms contained a large amorphous periodic acid-Schiff-negative inclusion that pushed cellular organelles to the periphery of the blastocidium. Some presented as "empty" cells with folded walls. Giant blastoconidia (saccules), inside of which several smaller blastoconidia were arranged in an orderly fashion, were observed rarely. The nature of the inducing agent(s) in chocolate agar has not as yet been identified. Because giant forms only develop on chocolate agar, some unique aspect of this medium is most likely responsible. Iso Vitalex, a vitamin and amino acid supplement of chocolate agar, does not induce giant cell production. Perhaps a component of the ruptures red blood cells comprising chocolate agar is responsible for inducing giant *C. albicans* blastoconidia. Giant blastoconidia represent a unique and previously undescribed form of *C. albicans.* Furthermore, the presence of internalized blastoconidia may represent an entirely new facet of the *C. albicans* life cycle, perhaps, necessitating a reclassification of the species.

The Effect of Yogurt on Antibiotic-Induced *Clostridium difficile*-related Diarrheal Illness in a Long-Term Facility. K. Chennapragada, V. M. K. Bhaskarabhatla, H. Rambaran, M. Green, B. Hauchwit, and N. Rambaran. Department of medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

As part of an ongoing study, we evaluated the effectiveness of lactinex or yogurt supplementation in reducing the incidence of antibiotic-induced *Clostridium difficile* diarrhea-related illness. It is well documented that patients treated with intravenous or oral antibiotics are at risk of developing diarrhea related to alteration of the gut flora with subsequent overgrowth of *C. difficile.* The study is being conducted in a long-term facility with a resident geriatric population of approximately 350 persons. For the preintervention group, data collection took place from July 1995 to June 1996 on all patients treated with antibiotics who developed diarrhea requiring stool culture and therapy. To examine the effect of lactinex or yogurt for the intervention group, data from July 1996 until February 1997 were collected for all patients to whom either yogurt or lactinex was administered along with each dose of antibiotics for the duratio;n of therapy. From July 1995 to June 1996, 52 patients developed diarrhea within 14 days after use of antibiotics. Stool examination showed *C. difficile* toxin A in all cases. Patients were treated with oral medications including vancomycin, metronidazole, and cholestyramine. Most patients had an uneventful recovery. From July 1996 to February 1997 (when yogurt or lactinex was instituted), there were 13 cases of toxin A-positive *C. difficile* colitis. Initial data analysis showed: pre-intervention, 52 cases/year; postintervention: 13 cases/6 months; ($p < 0.0001$). Our initial data analysis indicates that the use of yogurt or lactinex therapy is associated with a statistically significant decrease in the incidence of *C. difficile*-related diarrheal illness. We are currently planning a prospective therapeutic trial of these agents to further substantiate our findings.

Infectious Complications in Patients with HIV-Related Lymphoma. T. Cheung, S. Jones, and F. Wallach. Mount Sinai School of Medicine, New York, NY.

Objective: To evaluate the infectious and opportunistic infectious complications of patients with HIV-related lymphoma.

Methods: We evaluated retrospectively medical records of patients identified as having non-Hodgkin's lymphoma (NHL) and central nervous system lymphoma (CNSL) in our tumor registry and HIV (Jack-Martin Fund) clinic from 1990 to 1996. Charts of 24 patients with NHL and 20 patients with CNSL were reviewed.

Results: Patients with NHL had an age range of 20 to 61 years (median, 40 years); 17 men, 7 women; 19 Hispanics, 9 African Americans, 5 Whites. Their risk factors were 9 intravenous drug users (IVDU), 7 heterosexual, 6 homosexual, and 2 hemophiliac. The mean

CD4 counts were 49 ± 34 (10^6/L). Infectious complications (IC) were: 10 fever of unknown origin (FUO) (9 neutropenic), 3 line sepsis, 7 pneumonia, 6 sepsis (3 *Acinetobactor*, 2 *Candida*, 1 *Streptococcus viridans*), 1 *Pneumocystis carinii* pneumonia (PCP), 2 MTB, 5 *Candida* pharyngitis/esophagitis, 3 *Cytomegalovirus* (CMV) infection (2 retinitis, 1 colitis), 2 *Staphylococcus* cellulitis and 1 disseminated *mycobacterium avium-intracellulare* (MAI). The frequency was 0.45 episode/patient-month. Patients with CNSL had an age range of 32 to 51 years (median, 41 years); 15 men, 5 women, 14 Hispanics, 3 Whites, 3 African Americans, Mean CD4 counts were 20 ± 10 (10^6/L). Risk factors were 9 IVDU, 5 heterosexual, 4 homosexual, and 2 unknown. IC were: 6 FUO (3 neutropenic), 2 pneumonia, 2 PCP, 2 disseminated MAI, 3 urinary tract infectuibm 1 CNS toxoplasmosis. The frequency was 0.30 episode/patient-month. Patients with NHL have more LC. ($p < 0.05$, χ^2). The contributing factors were chemotherapy and neutropenia. A case-matched control study of these patients compared with other HIV patients without lymphoma is in progress.

Conclusion: Patients with NHL appear to have more IC than patients with CNSL, although patients with CNSL have a worse prognosis. The contributing factors were chemotherapy and neutropenia.

Liver Diseases

Functional Conservation of Hepatitis C Virus Proteins Is Greater Than Suggested by Conventional Genotyping. J. L. Walewski, D. D. Stump, T. R. Lefkowitz, W. Branch-Ellman, and A. D. Branch. Division of Liver Disease, Department of Medicine; Division of Abdominal Organ Transplantation, Department of Surgery; and Department of Microbiology, Mount Sinai School of Medicine, New York, NY.

Viral proteins play key roles in pathogenesis. Thus we are examining relationships among hepatitis C virus (HCV) isolates by comparing the protein sequences of individual HCV genes. The pairwise similarities are tested for agreement with those of conventional HCV genotyping, which is based on nucleic acid sequence analysis. This latter approach emphasizes lines of descent and divergent evolution, thereby potentially obscuring functional similarities.

Methods: 109 core and 103 Envelope 1 HCV sequences were retrieved from GENBANK. Pairwise comparisons determined the percent identity between sequences at both the nucleotide and amino acid levels [CLUSTAL, Megalign (DNAstar)].

Results: This analysis revealed that, in some cases, the amino acid sequences of isolates assigned to different genotypes are more similar to each other than to certain other isolates in the same genotype. For the core protein, two isolates of genotype 1b, S72727 and HPC1B4, are 88.5% identical in amino acid sequence, whereas the amino acid sequence of HPC1B4 is 89.5% identical with that of HPJ8G, which is in the genotype 2a (see table).

Conclusions: For HCV core and E1 genes, nucleic acid-based trees may exaggerate functional differences between some isolates and exaggerate similarities between others. To correlate HCV "genotypes" with disease manifestations, it may be necessary to divide HCV isolates into groups defined by their functional properties, rather than by ancestry, as reflected in their nucleic acid sequences. Additionally, nucleic acid-based similarities do not accurately predict amino acid similarities, which may further limit the usefulness of conventional genotyping in predicting disease outcome and/or appropriate treatment.

Gene region	Sequences compared	Nucleotide (%)	Amino acid (%)
Core	S72727 (1b) vs HPC1B4 (1b)	93.2	88.5
Core	HPC1B4 (1b) vs HPJ8G (2a)	78.6	89.5
Core	HCU10210 (3a) vs LK049E1 (10a)	82.7	93.2
Envelope 1	HPCEP1EK (1a) vs S71864 (1b)	64.9	71.4
Envelope 1	HPCEP1EW (1a) vs HPCEPE1P (5a)	58.3	70.8
Envelope 1	HPCPK6E (3a) vs HPCHCVT (3b)	67.2	71.9

Neoplastic Diseases

*†**Design and *in Vitro* Evaluation of Hairpin Ribozymes Against Deletion-Mutant Epidermal Growth Factor Receptor RNA for Prospective Glioblastoma Gene Therapy.** M.-E. Halatsch, I. C. Botefur, U. Schmidt, J. F. Holland, and T. Ohnuma. Division of Neoplastic Diseases, Department of Medicine, Mount Sinai School of Medicine, New York, NY.

Amplification of the epidermal growth factor receptor (EGFR) gene is present in about 50% of patients with primary glioblastoma multiforme (GBM), occurs almost exclusively in this type of brain tumor, and thus appears to be a late event in glial tumor progression. Although amplification of up to 12 additional genes including CDK4, MDM2, and SAS have been demonstrated in GBM, the EGFR gene remains the most commonly amplified gene in this tumor type. A significant fraction (about 50%) of the amplified genes are rearranged specifically to eliminate a DNA fragment containing exons 2–7 of the gene, resulting in an in-frame deletion of 801 bp (nt 275–1075) of the coding sequence of the extracellular domain. Although this truncated EGFR is constitutively (ligand-independently) activated and confers enhanced tumorigenicity *in vivo*, the underlying deletion is considered the most prevalent mutation involving the EGFR gene. Qualitative and quantitative aspects thus render this mutated receptor a potentially important therapeutic target. As a result of the deletion-mutation, the fusion-junction of the gene is created directly upstream of a ribozyme target codon (GUA). Ribozymes are catalytically active subsets of small RNA molecules which possess the dual properties of RNA sequence-specific recognition, analogous to conventional antisense molecules, and site-specific cleavage of RNA substrates. To moderate enhanced tumorigenicity of GBM mediated by amplification of deletion-mutant EGFR, we designed three different hairpin ribozymes against this target and evaluated their efficacy and specificity in a cell-free system. The ribozyme motifs were derived from the negative strands of satellite RNAs from tobacco ringspot virus (sTRSV), chicory yellow mottle virus (sCYMV1), and arabis mosaic virus (sArMV), respectively. DNA templates were synthesized by polymerase chain reaction (PCR) using sets of partial duplex-forming primers; each top primer contained the bacteriophage T7 RNA polymerase promoter sequence to allow for consecutive *in vitro* transcription of the PCR product. Synthetic EGFR substrates, both wild-type and deletion-mutated, were generated similarly. The sCYMV1 ribozyme was highly active and specifically cleaved its mutant substrate in time-, ribozyme/substrate ratio-, and magnesium concentration-dependent manners at pH 7.5 and 37°C. Under optimized conditions (12 mM $MgCl_2$, 1:1 ribozyme/substrate ratio), the extent of specific cleavage was >90% after 4 hours of incubation. The sCYMV1 ribozyme retained cleavage activity at 0.5 mM $MgCl_2$, which roughly equals the intracellular Mg^{2+} concentration; other divalent catalysts, e.g., Mn^{2+} and Zn^{2+}, may promote folding of a catalytically proficient ribozyme-substrate complex *in vivo*. In contrast, the sArMV attained <1% of cleavage activity under optimized conditions compared with the sCYMV1 ribozyme, whereas the sTRSV ribozyme was not active at all. None of the three ribozymes affected the wild-type EGFR RNA. These findings indicate that the sCYMV1 hairpin ribozyme may serve as a tool to specifically inhibit the expression of truncated EGFR and to potentially reverse the malignant phenotype of a molecularly defined subgroup of GBM. Evaluation of the effect of sCYMV1 in appropriate cell culture systems by using retrovirus-mediated gene transfer is currently in progress.

Induction of Apoptosis by Phenylbutyrate in Prostate Cancer Cells: Potential Pathways of Resistance of Androgen-Independent Cells. M. Gao, L. Ossowski, and A. C. Ferrari. Division of Neoplastic Diseases, Department of Medicine, Mount Sinai School of Medicine, New York, NY.

We investigated phenylbutyrate (PB), a novel antiproliferative and differentiation-inducing agent with a low toxicity profile, in phase I clinical trials for its potential therapeutic activity in advanced prostate cancer (PC). We used an androgen-independent (AI) derivative of the androgen-dependent (AD) prostate cancer cell line (LNCaP). Three days treatment with 1.5 mM PB was sufficient to induce 50% growth inhibition of AD cell; 5 mM PB was required to achieve the same effect in AI cells. At 5 mM concentration, apoptosis was detected by DNA ladder formation in the AD cells after 24 hours treatment but was delayed to 48 hours in the AI cells. To investigate the mechanism of action of PB in PC cells we studied the effect of 5 mM PB treatment on the expression of growth- and apoptosis-associated genes. Both cell lines showed decreased expression of the androgen receptor (AR) and reduction of the hyperphosphorylated retinoblastoma protein (pRb). The levels of Bcl-2 protein increased in both cell lines after treatment. The cyclin kinase inhibitor, WAF 1/p21 expression was transiently upregulated by PB treatment of AD cells but remained undetectable in AI cells. NFκB is a nuclear transcription factor whose activation can block apoptosis induction by tumor necrosis factor-α (TNFα). Treatment with 10 ng/mL TNFα (4 hours) or 5 mM PB (24 hours) was associated with activation of NFκB in AI cells but showed no effect on AD cells. These findings suggest that 1) AI cells are less sensitive to growth inhibition and induction of apoptosis by PB than AD cells; 2) extended treatment with PB can overcome resistance of AI cells; 3) down-modulation of the AR and pRB may be required for growth arrest and apoptosis by PB in AD and AI cells; 4) modulation of Bcl-2 does not appear to be associated with PB-induced apoptosis in either cell line; 5) WAF1/p21 may mediate the induction of apoptosis by PB in the AD cells; 6) resistance of AI cells may be related to the constitutive loss of WAF 1/p21 expression and the ease of activation of NFκB.

Induction of Apoptosis by Retinoic Acid in Androgen-Dependent LNCaP Cells and Its Androgen-Independent Derivative. M. Gao, L. Ossowski, and A. C. Ferrari. Division of Neoplastic Diseases, Department of Medicine, Mount Sinai School of Medicine, New York, NY.

Hormonal ablation is an effective therapy for most patients with prostate cancer. When tumors re-grow, they become androgen independent and resistant to all known therapies. We tried to mimic the evolution of androgen-independent (AI) cancer by chronically depriving an androgen-dependent (AD) prostate cancer cell line (LNCaP) of androgen, and after 5 months of such treatment, obtained an AI derivative. Unlike the AD cells, the AI cells did not produce prostate-specific antigen (PSA), and had ~4 times more androgen receptor mRNA and protein. In response to all-*trans*-retinoic acid treatment, known to induce differentiation in several cancer cell types, we found up-modulation of PSA and cytokeratin 18 in AD cells; PSA was undetectable in AI cells. Most importantly, an increased concentration of retinoic acid (RA; 2–5 mM) induced apoptosis in both AD and AI cells, but AI cells were much more resistant. Most AD cells were deemed apoptotic by three different methods (TUNEL, DNA ladder, and direct microscopic observation of DNA fragmentation) after 3 days of RA treatment, whereas AI cells required 7 days of treatment. The same was true when Taxol and Adriamycin were tested; AD cells underwent apoptosis after 1 day, whereas AI cells required 2 to 5 days of treatment, suggesting that like the recurrent cancer, these cells have an overall apoptosis-resistant phenotype. We investigated the mechanism of this relative resistance of AI cells and found that they expressed higher (2- to 4-fold) constitutive levels of Bcl 2 protein. Examination of Rb protein showed a predominance of the underphosphorylated form in AD cells treated with RA for 1 day. A much longer treatment was required for the AI cells. In both cell types a reduction in AR level preceded apoptosis. It appears that the pathway leading to RA-induced apoptosis involves Bcl2 and AR and that AI cells may be more resistant because of the overexpression of these two proteins.

***WT1* Expression Induces Features of Renal Epithelial Differentiation in Mesenchymal Fibroblasts.** S. Hosono, X. Luo, D. P. Hylink, L. M. Schnapp, P. D. Wilson, C. R. Burrow, G. F. Atweh, and J. D. Licht. Brookdale Center for Developmental and Molecular Biology and Department of Medicine, Mount Sinai School of Medicine, New York, NY.

The *WT1* tumor suppressor gene, implicated in hereditofamilial and sporadic Wilms' tumor, is required for normal renal development and is up-regulated during the mesenchymal-epithelial transition. NIH 3T3 fibroblasts overexpressing *WT1* are less proliferative, larger in size, and attach more tightly to plastic, suggesting an alteration of their differentiation state. These cells were studied *in vivo* by injecting large numbers subcutaneously into nude mice. The resulting tumor exhibited epithelioid histopathology and formed desmosome-like structures. Molecular analyses of these *WT1* expressing fibroblasts *in vitro* and *in vivo* revealed significant alterations in the expression of many markers of kidney epithelial differentiation. These studies suggest a key role for *WT1* in the mesenchymal-epithelial transition that occurs during renal development and suggest several novel target genes for the WT1 transcription factor, including uvomorulin, integrin α8, and type IV collagen, and suggest that WT1 may activate the IGF-II gene also implicated in the development of Wilms' tumor.

Failure of Downstream Processes at M-phase Checkpoint in Tumor Cells Induced *In Vitro* by a Newly Synthesized Antimicrotubule Agent 3-Bromoacetylamino Benzoylurea. J. D. Jiang, J. Strauchen, J. Weisz, J. F. Holland, and J. G. Bekesi. Division of Neoplastic Diseases, Department of Medicine, Mount Sinai School of Medicine, New York, NY.

We have synthesized a group of compounds exhibiting inhibitory activities for microtubule assembly in tumor cells, thereby inducing mitotic arrest and leading to cell death. 3-Bromoacetylamino benzoylurea (3-BAABU) showed strong cancericidal activity via inducing mitotic arrest and subsequently apoptosis in human T-cell leukemic cells (CEM), human biphenotypic leukemic cells (SP), human prostate cancer cell line (PC-3), murine melanoma cells (B-16) and murine lymphoma/leukemia cells (EL4) *in vitro* with an ID$_{50}$ in the 0.013–0.07 μg/mL (0.04–0.22 μM) range. Treatment of tumor cells for 12 to 24 hours with 3-BAABU resulted in mitoric arrest at premetaphase/metaphase/anaphase with separation and dispersion of chromosomes, and with the absence of mitotic spindle apparatus. Cell cycle analyses showed that most of the treated leukemic cells were accumulated at M-phase 12 hours after treatment. By the end of 48 hours of treatment the cells underwent apoptosis with nuclear condensation and DNA fragmentation. Furthermore, we found that 3.-BAABU inhibited the assembly of microtubules from tubulin, but did not interfere with the disassembly of microtubules. Substituting the Br with Cl yielded 3-chloroacetylamino benzoylurea which had much less mitotic blocking activity in tumor cells and increased the ID$_{50}$ 50-fold. Substitution of the urea group with ethyl ester abrogated the activity of blocking mitosis but induced apoptosis. Moving the bromoacetylamino group from the meta- to the para-position yielded 4-bromoacetylamino benzoylurea which had no blocking activity for mitosis but induced necrosis. Therefore, the presence and the position of Br and urea groups on the benzoic ring are determinant factors for its inhibition of microtubule assembly. Treatment with 3-BAABU had no cytotoxic and mitotic blocking effect in normal human lymphocytes, proliferating fibroblast cells (3T3), and proliferating myocardial cells (MOT). These data suggest that 3-BAABU is a potential agent for cancer chemotherapy.

Identification of Retroviral Sequences in Human Breast Cancer. B. Liu, Y. Wang, I. Pelisson, S. M. Melana, J. F. Holland, V. Najfeld, and B. G.-T. Pogo. Divisions of Neoplastic Diseases and Hematology, Department of Medicine, Department of Pathology and Department of Microbiology, Mount Sinai School of Medicine, New York, NY.

We have previously shown that a 660-bp sequence homologous to the *env* gene of the mouse mammary tumor virus (MMTV), but not to the endogenous retrovirus HER-K10, can be detected by polymerase chain reaction (PCR) in 38% of human breast cancers and in 1.8% of normal breasts. Using long PCR we now report the identification of a 2.7-kbp sequence comprising the LTR and *env* genes and a 1.6-kbp sequence comprising LTF and *gag* genes. Both were highly homologous to MMTV, but not to HER-K10. In addition, a 3.1-kbp sequence comprising *gag-pol* genes and an 800-bp sequence comprising the *env-pol* junction were also amplified and sequenced. Both showed high homology to MMTV, and not to extended regions of HER-K10. Using the 2.7-kbp clone as a probe it was possible to perform fluorescence *in situ* hybridization analysis and localize the sequences in prophase nuclei and in chromosomes of breast cancer cells *in vitro*, but not in normal breast cells. These findings support the proposition that a substantial portion of human breast cancer is associated with sequences homologous to MMTV which appear to be part of a human MTV.

Evaluation of the Antiviral Nucleoside Penciclovir in the HSV-tk/Ganciclovir Suicide Gene Therapy System. J. C. Morris,[1,2] J. Higginbotham,[2] O. Wildner,[2] W. J. Ramsey,[2] D. Bond,[2] S. E. Straus,[3] and R. M. Blaese.[2] [1]Division of Neoplastic Diseases, Mount Sinai School of Medicine, New York, NY; [2] Clinical Gene Therapy Branch, National Human Genome Research Institute; and [3]Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, Bethesda, MD.

Ganciclovir (GCV) is able to kill cells expressing the *Herpes simplex* virus-thymidine kinase (HSV-tk) gene. The mechanism of GCV cytotoxicity is based on its relatively specific phosphorylation by HSV-tk and its subsequent inhibition of cellular DNA synthesis. The HSV-tk/GCV "suicide" gene therapy system is under extensive investigation as a novel anticancer treatment. The prodrug GCV, however, is inherently bone marrow toxic and also requires intravenous administration. The recent development of several other antiviral nucleoside analogs may offer less toxic and more convenient alternatives to GCV. Penciclovir (PCV) is less toxic to tk cells than GCV, it is rapidly phosphorylated by HSV-tk, and the active phosphorylated forms have half-lives similar to those of GCV. PCV can achieve serum levels similar to GCV. In addition, PCV has an available oral prodrug (famciclovir). To test its activity, tk$^+$ retroviral transduced (STK) murine Neuro-2a cells, MCA 38 cells, rat 9L gliosarcoma cells, and human A375 melanoma, HeLa cells, and K562 leukemia were treated with PCV. Cytotoxic effects of PCV and GCV were compared by ^{3}H-thymidine (^{3}H-Tdr) incorporation assay. At equimolar concentrations, GCV was the more active agent; however, when compared at clinically relevant doses, inhibition of ^{3}H-Tdr labeling was similar with both drugs. Both drugs resulted in similar decreases of ^{3}H-Tdr uptake in cell lines transduced with a tk$^+$-expressing adenovirus. Intraperitoneal administration of PCV induced regression of subcutaneous tk$^+$ MCA 38STK colon carcinoma tumors in C57/B6 mice. Studies to define the appropriate *in vivo* dosing of PCV and high-performance liquid chromatography analysis of the intracellular concentrations and half-life activity of the phosphorylated intermediates are in progress.

Conclusion: Newer antiherpetic nucleosides may have efficacy similar to GCV in the HSV-tk system and more cost effective.

Induction of Morphological and Biochemical Changes in Prostatic Carcinoma Cell Lines by a Pituitary Differentiation Factor. M. Platica, M. Lopingco, J. Holland, and O. Platica. Mount Sinai School of Medicine, New York, NY; and VA Medical Center, Brooklyn, NY.

We demonstrated previously that an alkaline pituitary extract (PE) from the mammosomatotrophic tumor MtTW10 induced differentiation of prolactin-independent rat mammary tumor cells MTW9/PL. In our current study, we analyzed changes in morphology and gene expression in response to PE by four human prostatic carcinoma cell lines: DU 145, from a brain metastasis; PC3, from a bone metastasis; LNCaP.FGC, from a lymph node metastasis; and LNCaP-OM, a hormone-independent derivative of LNCaP.FGC. PE induced various degrees of cell aggregation and spheroid formation in all four cell lines. In contrast, untreated control cells remained dispersed. A linear correlation between the concentration of PE and aggregation was also observed. To measure changes in steady state RNA levels of E-cadherin and prostate-specific antigen (PSA), we used quantitative reverse transcriptase-polymerase chain reaction. An increase in E-cadherin expression appears to parallel the PE-induced cell aggregation. Because decreased E-cadherin is usually associated with progression to a malignant phenotype, the increased level of E-cadherin induced by PE may reflect the conversion to a more benign, less aggressive pattern of growth. PSA mRNA was increased similarly and may indicate progression toward a more differentiated phenotype. Using an expression-cloning strategy, we recently identified a clone, pituitary differentiation factor (PDF) from a human pituitary carcinoma library that most likely is responsible for this differentiation activity. Based on preliminary sequence analysis, it appears that PDF is a novel protein. Studies aimed at characterizing and elucidating the mechanism of action of PDF are currently in progress.

Selective Tumor Apoptosis by MF13, L-prolyl-L-*m*[bis(chloroethyl)amino]-phenylalanyl-L-norvaline ethyl ester, a New Sarcolysin-Containing Tripeptide. J. Roboz, J. Jiang, I. Weisz, F. Holland, and J. G. Bekesi. Division of Neoplastic Diseases, Department of Medicine, Mount Sinai School of Medicine, New York, NY.

ID$_{50}$ and ID$_{90}$ of L-prolyl-L-*m*-[bis(chloroethyl)amino]-phenylalanyl-L-norvaline ethyl ester HCl, MF13, were determined in four murine (leukemia, lymphoma, melanoma, lung) and eight human cancer cell lines (two leukemia, prostate, kidney, colon, two melanoma, breast). Cytotoxic activity was two to five times higher than that of sarcolysin {(L-3-[bis(2-chloroethyl)amino]-L-phenylalanine} against all leukemias and lymphomas, ID$_{50}$ 0.5–0.9 μM, and against human solid tumors, ID$_{50}$ 0.4–2.1 μM. Sensitivities of L-PAM-resistant and methotrexate-resistant L1210 cells were the same as the naive lines, ID$_{50}$ 0.5 μM. Apoptosis was confirmed by: (a) morphology, revealing chromatin condensation and nuclear fragmentation; (b) flow cytometry, showing changes in cell size and DNA integrity; (c) DNA electrophoresis, demonstrating multiples of 180–200 bp DNA units. MF 13 had no cytotoxicity against human peripheral blood lymphocytes at concentrations lethal to tumor cells (ID$_{50}$ 13.3 μM without and 11 μM with phytohemagglutin stimulation), and failed to induce apoptosis. Subcutaneous MF 13 treatment of mice with advanced EL4 leukemic ascites yielded extensive apoptosis with DNA degradation identical with that seen *in vitro*, and resulted in complete tumor regression in all treated mice. These results suggest MF 13 as a potential chemotherapeutic agent.

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Monitoring the Stepwise Decomposition of Hyaluronan by Endoglycosidases with Negative Electrospray Mass Spectrometry. J. Roboz, L. Ma, L. Deng, and T. Wang. Division of Neoplastic Diseases, Department of Medicine, Mount Sinai School of Medicine, New York, NY.

This study used negative electrospray (ESI) mass spectrometry to monitor the stepwise decomposition of hyaluronan (HA, hyaluronic acid) incubated with hyaluronidase (H'ase) in serum and effusates from patients with malignant mesothelioma. Standard HA was partially purified by high-performance liquid chromatography (HPLC). Samples from patients with mesothelioma were incubated with H'ase and injected directly, or injected after isolation of HA using size exclusion (5 kDa) HPLC. Incubations with H'ase were carried out at 37°C for a variety of times, from 5 minutes to 4 days, using a range of substrate/enzyme concentration ratios. Using negative ESI optimized sensitivity for high molecular weight oligosaccharides and eliminated interferences by H'ase and major serum constituents because of poor sensitivity to proteins. The disaccharide in HA was D-glucuronic acid + 2-acetamido-2-deoxy-D-glucose ($\approx$3000 disaccharide units, 379 Da each) yielding a molecular mass of approximately 1.1 MDa. The highest detectable mass was 205.2 kDa or 542 disaccharide units. Despite polydispersity, the decomposition could be followed stepwise even in direct injection of mesothelioma serum incubates. H'ase action on HA occurred both at the end of chains and inside the chains. Endproducts were tetra- and hexasaccharides. Although the decomposition behavior of cord and mesothelial patient HA was similar, the detected differences suggest higher polydispersity of HA from patients.

Conclusions: (a) Advantages of negative ESI included sensitivity to oligosaccharides, but insensitivity to H'ase. (b) In addition to removing disaccharides stepwise, H'ase also made scissions in the middle of the chains and gave hydrated di- and tetrasaccharide products. (c) Decomposition of polydisperse HA by H'ase in mesothelioma serum could be monitored.

This work was supported by T. J. Martell Foundation for Leukemia, Cancer and AIDS Research.

Overexpression of Epidermal Growth Factor Receptors and MDM2 Are Independent Genetic Events in Human Glioblastoma Multiforms. U. Schmidt, M.-E. Halatsch, and T. Ohnuma. Division of Neoplastic Diseases, Department of Medicine, Mount Sinai School of Medicine, New York, NY.

Because 75% of all patients with glioblastoma multiforme (GBM) die within 18 months of diagnosis, this type of tumor is among the most devastating of human malignancies. Although primary GBM predominate and arise rapidly in a *de novo* fashion (i.e., without demonstrable precursor lesions), secondary GBM evolve through progression from lower-grade astrocytomas. Different molecular pathways including amplification and activation of cellular oncogenes as well as inactivation of tumor suppressor genes have been identified for both types of GBM. The most common amplification target in primary GBM is the epidermal growth factor receptor (EGFR) gene which is amplified in about 50% of patients with this type of tumor. A significant fraction (about 50%) of the amplified genes are rearranged specifically to eliminate a DNA fragment containing exons 2–7 of the gene, resulting in an in-frame deletion of 801 bp (nt 275–1075) of the coding sequence of the extracellular domain. This truncated EGFR is constitutively (ligand-independently) activated and has been shown to confer enhanced tumorigenicity *in vivo*. Although EGFR amplification and truncation are largely limited to primary GBM, inactivation of the tumor suppressor gene p53 by mutation or allelic loss involving chromosome 17p is essentially restricted to the secondary glioblastoma pathway. Therefore, EGFR amplification and mutation of p53 are considered mutually exclusive in GBM. Recently, evidence suggests that amplification of the mouse double minute 2 (MDM2) gene, which acts as a negative cellular regulator of p53, may abrogate wild-type p53 tumor suppressor gene function in primary GBM. Inactivation of p53 occurs through formation of oligomeric complexes between the MDM2 gene product and p53 protein. This molecular mechanism apparently allows primary glioblastomas typically lacking p53 mutations to escape from p53-regulated growth control. In this study, we evaluated the relationship between overexpression of wild-type or deletion-mutant EGFR and MDM2 using the wild-type EGFR/wild-type p53-expressing human glioblastoma cell line U-87MG as well as the related cell lines U-87MG.wtEGFR and U-87MG.ΔEGFR, which were generated by retroviral infection of the parental U-87MG cell line and overexpress exogenous wild-type (wt) or deletion-mutant (Δ) EGFR. Total cellular RNAs were isolated from these cell lines, treated with DNase, and subjects to reverse transcription (RT) using oligo(dT) primers. The resulting cDNAs were amplified by differential display-polymerase chain reaction (RT-DD-PCR) using two sets of primers for the MDM2 target gene and the β-actin reference gene, respectively. Parts of the RT-DD-PCR reaction mixtures were separated on ethidium bromide-stained agarose gels which yielded two sharp and distinct bands in accordance with the predicted sizes of the PCR products (MDM2: 143 bp; β-actin: 661 bp); photographs of the gels were assessed for differences in band intensities. Although the β-actin band was stronger than the MDM2 band in each cell line, there was no significant variance when the bands pertaining to either MDM2 or β-actin were compared among the cell lines. We conclude that overexpression of wild-type or deletion-mutant EGFR does not influence MDM2 expression and that these occurrences constitute independent genetic events in the evolution of human GBM.

Computed Tomography-Guided Percutaneous Ethanol Injection Therapy for Large Hepatocellular Carcinoma. S. H. Sigal,[1] I. B. Denenberg,[2] and A. J. McElhinney.[3] [1]Alcohol Research Program, [2]Radiologic, and [3]Surgical Services, Bronx VA Medical Center, New York, NY.

Despite recent progress, treatment of hepatocellular carcinoma (HCC) is limited. For small lesions, resection, percutaneous ethanol injection therapy (PEIT), and orthotopic liver transplantation are effective. Available therapy for large, nonresectable lesions, however, is suboptimal. PEIT under ultrasound guidance is ineffective for lesions greater than 2 cm because of the limited diffusion of ethanol and difficulty in ensuring complete treatment of the lesion. In this report we describe the effective application of PEIT under computed tomography (CT) guidance for a large (6 cm), unresectable HCC in a 68-year-old man who presented with abnormal liver tests associated with cirrhosis from alcohol and chronic hepatitis C with an α-fetoprotein (AFP) level of 844 ng/mL in December 1996.

Methods: After determining the location of the tumor on abdominal CT scanning with i.v. contrast, PEIT was performed under CT guidance using a 22-gauge needle. Except for one session in which i.v. contrast was first administered, cysts adjacent to the tumor served as landmarks for needle localization. After documentation of intratumor administration with a 1-mL injection, 2- to 3-mL aliquots of ethanol for a total of 8–10 mL per session were administered. After each injection, confirmation of injection within the tumor was determined by CT scanning. After the last injection, the needle was cleared of ethanol with lidocaine before its removal. PEIT was performed every 2–3 weeks, and CT scan with i.v. contrast was performed after every two sessions to locate remaining areas of viable tumor. AFP levels to estimate tumor burden and liver function tests were monitored during treatment.

Results: CT scan with i.v. contrast revealed an enhancing 6-cm lesion located in the medial aspect of the left lobe surrounded by cysts which was not identifiable on noncontrast examination. CT scanning immediately after injection of ethanol revealed an area of very low attenuation with cystic characteristics of a volume corresponding to the amount injected. Serial CT scans with i.v. contrast between PEIT sessions revealed an area of homogeneous, low attenuation corresponding to necrotic tumor in the areas injected with adjacent areas of enhancement consistent with viable tumor. The enhancing regions were then injected during subsequent treatments. The patient tolerated the procedure well with minimal discomfort at the time of removal of the needle. Initially, he was admitted to the hospital overnight for observation. However, after the first few sessions PEIT was performed on an ambulatory basis. After administration of a total of 67 mL during eight sessions between March and July 1997, the entire lesion was treated.

Liver function remained stable as AFP level progressively decreased to 87 ng/mL.

Conclusions: CT-guided PEIT is effective treatment for large HCC as determined by serial CT scanning and AFP levels. It can be performed easily on an ambulatory basis with minimal discomfort without deterioration in liver function in patients with nonresectable lesions and advanced cirrhosis. Interval CT scanning with i.v. contrast is helpful in indicating areas of remaining viable (enhancing) tumor needing treatment.

New Periadjuvant Treatment Strategies for Gastric and Gastroesophageal Junction Cancers. N. S. Turhal,[1] J. Dalton,[2] and H. W. Bruckner.[1] [1]Division of Neoplastic Diseases, Department of Medicine, and [2]Department of Radiation Oncology, Mount Sinai School of Medicine, New York, NY.

Objective: Evaluation of a flexible, multistep approach for periadjuvant therapy of gastric and gastroesophageal (GE) junction adenocarcinoma. The plan incorporates new drugs such as methotrexate (M) and hydroxyurea in addition to leucovorin (L), fluorouracil (F), and cisplatin (P) and split-course radiotherapy schedules.

Methods: From December 1989 to October 1994, 11 patients with clinically T3–4, NX, or 1 and M0 tumors with biopsy-proven adenocarcinoma of the stomach or GE junction crossing the Z line were treated with combined modality radiotherapy and chemotherapy (PLF, MLF or MLPF depending on stage and location of the tumor and the age and status of patient.

Results: Combined modality periadjuvant therapies downsized 10 of 11 T3–4 gastric and GE junction cancers and produced four pathologically proven complete remissions. Three patients had minute foci of occult metastatic disease. Treatment converted lymph nodes to a cancer-free status for seven of eight evaluable patients. The quality of the responses was underestimated by both endoscopic ultrasound and CT scans. Responses were sometimes only achieved after 2 to 3 months of therapy. Modest initial responses were converted to complete remissions with radiotherapy and/or change of chemotherapy for 3 of the 11 patients. Median survival exceeds 2 years.

Conclusion: A flexible, multistep approach to periadjuvant therapy of gastric and GE junction cancers is well tolerated and results in higher rates of response, longer survival, and better quality of life than reported in parallel trials. Long delays in surgery (gastrectomy) appear to be safe in the context of combined modality therapy (CMT). The experience suggests new early endpoints for evaluation of periadjuvant treatments: quality of life, quality of lymph node sterilization, and extent of required gastrectomy in comparison with standard surgery or CMT. Radiotherapy based on site of failure and response to second treatment appears to be an important component for successful outcome.

Retrovirus-Mediated Anti-*MDR*1 Ribozymes Fully Restore Chemosensitivity of P-Glycoprotein-Expressing Human Lymphoma Leukemia Cells. F. S. Wang, J. F. Holland, and T. Ohnuma. Division of Neoplastic Diseases, Department of Medicine, Mount Sinai School of Medicine, New York, NY.

Overexpression of MDR1 gene is a major mechanism of multidrug resistance (MDR) which seriously limits the efficacy of anticancer agents. Thus, circumvention of MDR is one of the urgent goals in cancer chemotherapy. One strategy to block the overexpression of MDR1 is the use of a ribozyme (Rz) targeted against MDR1 mRNA. We developed Daudi human lymphoma cells that are 20-fold resistant to vincristine (VCR) by infecting cells with pHaMDR1/A retroviral vector (Dr. Gottesman) (Daudi/MDR/VCR_{20}). This resistance was purely related to P-glycoprotein (P-gp) expression. Three DNA sequences of anti-MDR1 hammerhead Rz genes—one cleaving codon 196 of MRD1 mRNA (196-MDR1 Rz); the second, a stem-II base-modified ($U_9 \rightarrow G_9$, $U_{13} \rightarrow A_{13}$, $G_{14} \rightarrow A_{14}$, $A_{18} \rightarrow C_{18}$) Rz against codon 196 (196-MDR1-sRz); and the third, the stem-II base-modified Rz (iMRD1-sRz) directed against the –6 ~ –4 GUC sequence of the translation initiation site of the MDR1 mRNA—were synthesized and cloned into the retroviral vector N2A+$tRNA_i^{met}$ downstream of tRNA gene sequence behind the RNA polymerase III promoter, forming the constructs: N2A+$tRNA_i^{met}$-196-Rz, N2A+$tRNA_i^{met}$-196-sRz, and N2A+$tRNA_i^{met}$-iMDR-sRz. In cell-free system, the chimeric tRNA/Rz molecules showed efficient catalytic activities compared with simple Rz molecules, and the stem II base-modified iMDR-sRz and 196-MDR-sTz were able to cleave completely the MDR1 substrate RNAs (Rz/substrate ratio, 2:1) at 37°C within 12 hours incubation. The three constructs were transfected into GP+env AM12 cells for packaging the retroviral vectors inserted with ribozyme genes. The supernatant containing the packaged retrovirus in high titers ($1.1–2.5 \times 10^5$ CFU/mL determined by NIH3T3 cells) were used to infect 1×10^6 Daudi/MDR/VCR_{20} cells. After G418 selection, all the Rz-transduced Daudi/MDR/VCR_{20} cells were evaluated for reversal of the MDR phenotype by cytotoxicity assay. The iMDR-sRz and 196-MDR1-sRz-transduced Daudi/MDR/VCR_{20} cells completely restored chemosensitivity to VCR, which was accompanied by the blocked expression of MDR1 mRNA and P-gp as well as increased expression of anti-MDR1 Rz.

In conclusion, the base modifications in the Rz stem II structure and the development of chimeric tRNA/Rz molecules were identified as enhancements of the cleavage efficacy, a fact likely associated with the stabilization of Rz structure. These refinements of anti-MDR1 Rz strategy should lead to the promise of more effective chemotherapy for patients with cancer.

Nephrology

Elevation of Venous Chamber Pressure, Access Stenosis, and Prediction of Access Failure by a Noninvasive Test in Patients Receiving Hemodialysis. F. J. Amede, D. Y. R. Adib, K. E. Vitting, V. A. DeBari, and J. R. Sterrett. Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

Patients receiving hemodialysis require permanent access, either an endogenous arteriovenous fistula (AVF) or a synthetic arteriovenous graft (AVG). Complications account for a significant percentage of hospital admissions. We investigated the relationship between persistent elevated venous chamber pressure (↑VCP) >100 torr, access stenosis (AcS), and potential risk factors (PRF) for access failure. A retrospective case-control study was conducted in which VCP at a blood flow (Qb) of 200 cc/min was assessed during 10 consecutive HD sessions in two groups, the study group with AcS (+AcS), documented by fistulography ($n = 20$), and a control group presumed without AcS (−AcS) ($n = 19$). PRF were: presence of AVG vs AVF, diabetes (DM), and hypertension (HTN). The −AcS group had good access function for 12–240 months ($\bar{x} = 58.3 \pm 59.9$), no access failures, nor ↑VCP. The +AcS group had access failures, and ↑VCP. These were analyzed by a contingency table model of logistic regression. ↑VCP (mean $\bar{x} \pm$ SD in torr) for the +AcS group was significantly higher than that for the −AcS group (123 ± 20 vs 74 ± 20; $p < 0.0001$). Longitudinal variability (assessed by variability index) was not different for the groups (+AcS group: 1.6 ± 0.7 vs −AcS group: 1.5 ± 0.7, $p = 0.88$). Patients with +AcS tended to have higher incidence of DM and HTN (nonsignificant). A significantly greater proportion of the +AcS group had AVG vs the −AcS group (90% vs 11%; $p < 0.0001$). (1) A consistent ↑VCP (>100 torr at Qb 200 cc/min) is a predictor of access stenosis or potential thrombosis/failure. (2) +AcS is more likely to be associated with AVG vs AVF. (3) A prospective cohort study of the incidence of AcS and subsequent access failure at normal VCP and ↑VCP should be undertaken.

The Sodium-Independent K-Cl Cotransporter Is Highly Expressed in the Human Kidney. D. M. Kaji, M. K. Nag, and H. Liapis. Renal Division. Bronx VA Medical Center Mount Sinai School of Medicine, New York, NY; and Department of Pathology, Washington University School of Medicine, St. Louis, MO.

The K-Cl cotransporter (KCCl) is a Na-independent ion transport pathway that plays an important role in volume regulation in many different cell types. Indirect evidence from previous workers has suggested that KCCl may play an important role in epithelial potassium transport in the kidney. By Northern blot analysis, the KCCl mRNA was found recently to be abundant in the kidneys, lungs, liver, and intestines, but the precise cell types that express KCCl in the human kidney and the role of KCCl in the human kidney remain unknown.

In this study, we describe the intrarenal distribution of KCCl. Sections were prepared from formalin-fixed paraffin-embedded blocks of surgically removed human kidney. A 540 nucleotide, ^{35}S, and/or digoxigenin-labeled anti-sense KCCl cRNA was used to perform *in situ* hybridization to characterize the cellular distribution of mRNA encoding the K-Cl cotransporter KCCl. A sense probe was used as a control.

Intense KCCl staining was detected in distal convoluted tubular epithelium, in the glomerular mesangial cells, and in the smooth muscle cells of the interlobular arteries. Only weak staining was observed in the proximal tubular cells, and in the interstitial fibroblasts.

The abundant expression of K-Cl cotransport mRNA in the distal tubular epithelial cells suggests an important role for the K-Cl cotransporter in distal tubular K handling and perhaps in volume regulation.

Development and Progression of HIV-Associated Nephropathy in Transgenic Mice Is Influenced by Genetic Background of Mice. S. Oller, A. Gharavi, L. A. Bruggeman, R. P. Lifton, and P. E. Klotman. Department of Nephrology, Mount Sinai School of Medicine, New York, NY; and Department of Medicine and Genetics, Yale University, New Haven, CT.

Clinical and histological features of HIV-associated nephropathy (HIVAN) were shown in an HIV-1 transgenic mouse in previous studies. Further studies have shown that the penetrance of renal disease has increased significantly through inbreeding. This suggests that there are genetically linked risk factors associated with HIVAN. In contrast to the out-bred line, 100% of the animals develop renal disease, and 75% of the animals have end-stage renal failure by 100 days of age. Because the transgene insertion site and the expression remained unchanged, it is hypothesized that inbreeding selects for specific genetic factors which predispose to the development of renal disease. To test this hypothesis and understand the influence of genetic factors on renal pathology, we have bred the transgene on different strains of mice and compared the rate of renal failure and the renal pathology to the original background (FVB/N). Cross-breeding studies onto the SJL and C57BL/6 backgrounds have reproduced many aspects of the disease that correlate with the original background (FVB/N) as well as differ in specific areas. Histological comparison of the SJL background indicated more extensive tubular epithelial cell proliferation and cell death by apoptosis. These studies denoted that both the progression of focal segmental glomerulosclerosis and tubulointerstitial disease, as well as the development of renal pathology, are influenced by genetic risk factors. These findings further imply that the development and progression of HIVAN may be affected by these genetic risk factors in humans.

Carboxyhemoglobin Levels in a Surgical Intensive Care Unit. M. Sedlacek,[1] J. Uribari,[1] W. Cobb,[2] B. Seabrook,[2] and N. A. Halpern.[3] [1]Division of Nephrology, Mount Sinai School of Medicine, New York, NY; and [2]Surgical Intensive Care Unit and [3]Department of Medicine, Bronx VA Medical Center, NY.

Endogenous carbon monoxide (CO) is produced by heme oxygenase isoenzymes in the degradation of heme-containing molecules. Recent studies have shown that hypoxia induces heme oxygenase production of CO in cultured vascular smooth muscle cells.

Because plasma lactate levels are used to define tissue hypoxia, we decided to determine whether there was any correlation between lactate and CO levels in a group of critically ill patients with a high likelihood of hypoxia. We assumed that CO produced endogenously in the blood vessel wall is bound by circulating hemoglobin and can be measured as carboxyhemoglobin (COHb).

Methods: All arterial blood gas, COHb, electrolyte and lactate levels routinely measured with a CIBA-Corning 865 system in the Bronx VA Surgical Intensive Care Unit from November 8, 1996 to June 28, 1997 were reviewed, and correlation analysis was performed with a Stat view 6.01 program.

Results: Of 183 patients 61% had elevated lactate levels (>2.5 mM/L) and 46% had elevated COHb levels (>1.5%). Lactate levels ranged from 0.12 to 2.69 and COHb levels from 0 to 4.8%. Fourteen (78%) of 18 patients with a lactate greater than 5 mM/L also had elevated COHb levels during their stay in the Intensive Care Unit. However, the correlation coefficient between lactate and COHb calculated on 5322 simultaneous measurements was 0.07 with $p < 0.001$.

Conclusion: Although elevated COHb levels may sometimes be observed in critically ill patients, they do not correlate with lactate levels taken as an indicator of tissue hypoxia. Our *in vivo* study does not support the proposed effect of hypoxia to increase endogenous carbon monoxide production. Thus the physiologic role of CO remains controversial; this may be partly because of the nonspecific effects of SnPP and other metalloporphyrins used to inhibit heme oxygenase in *in vitro* experiments.

Defense of Total and Free Intracellular Magnesium in Patients with Normal Renal Function and with End-State Renal Disease Receiving Dialysis. W. Shikoff and D. Kaji. Renal Division, Bronx VA Medical Center and Mount Sinai School of Medicine, New York, NY.

Chronic renal failure is associated with hypermagnesemia and elevated total Mg content of tissues. In the present study, we examined the plasma total Mg, cell total Mg (Mg_c), and free intracellular Mg ([Mg_i] in 10 control subjects with normal renal function and 20 patients with ESRD receiving maintenance dialysis. Free intracellular Mg was determined on polymorphonuclear leukocytes (PMNL) by ratiometric fluorescent dye furaptra. Each measurement was an average of 10 to 15 determinations, each derived from 2 to 3 PMNLs.

Mean total plasma Mg was normal in ESRD patients, but three patients had frank hypermagnesemia at the time of the study. PMNL cytosolic Mg was 0.52 ± 0.05 mM in ESRD patients vs 0.49 ± 0.04 mM in controls (NS). Cytosolic Mg was also not elevated in the three patients with hypermagnesemia (0.55 + 0.05 mM).

To better understand how Mg_c and $[Mg]_i$ change in response to extracellular Mg, we incubated PMNs from healthy subjects and from ESRD patients in media containing 0.6 (physiological), 2.5, 5, and 10 mM free Mg. Total cell Mg rose minimally (~10% above basal values), whereas free intracellular Mg remained unchanged at all Mg levels in PMNs from healthy subjects and from ESRD patients. When cells were incubated with the same high free extracellular Mg in the presence of an ionophore (10 mM A23187), total cell Mg content rose progressively in both groups, and was significant at 5 and 10 mM external Mg. However, intracellular Mg rose less than 10% above baseline, both in PMNs from healthy subjects and PMNs from ESRD patients.

Thus, cell Mg is tightly controlled both in healthy subjects and in ESRD patients. In cells exposed to high external Mg, total cell Mg, and free intracellular Mg are both normal, suggesting an important role for Mg extrusion transport pathways. In cells exposed to high external Mg and ionophore, total cell Mg rises, suggesting that transport pathways that extrude Mg from the cell are overwhelmed. However, free cell Mg is maintained at near-normal levels, suggesting that mechanisms that transport Mg from the cytosol to the intracellular organelles are activated to keep $[Mg]_i$ within the normal range. The transport mechanisms that transport Mg from the cytosol across plasma membrane and from cytosol to the organelles also explain why $[Mg]_i$ is not elevated in ESRD despite hypermagnesemia.

HIV-Associated Nephropathy is a Late, Not Early, Complication of HIV-1 Infection. J. Winston, A. Fozailoff, M. Klotman, and P. E. Klotman. Divisions of Nephrology and Infectious Diseases, Mount Sinai School of Medicine, New York, NY.

Renal disease in HIV-1 seropositive patients is usually caused by HIV-associated nephropathy. Previously, the development of nephropathy had been reported to occur at any stage of HIV-1 infection, but recent clinical trends indicate that this view may need to be revised.

We studied patients with biopsy-proven HIV-associated nephropathy (HIVAN) and seropositive patients who progressed to end-stage renal disease (ESRD) since 1995. We determined whether AIDS, as defined by the 1993 Centers for Disease Control and Prevention (CDC) case definition, was present at the onset of nephropathy, and whether AIDS developed before or after the onset of renal failure. All patients with biopsy-proven HIVAN during the past 18 months had CD4 counts below 200/mm^3 (n = 11). Since 1995, 352 patients began ESRD therapy and 19 were HIV-1-seropositive (5.4%). CD4+ counts before initiating dialysis were available in 17 patients. Mean CD4 count was 77 ± 29/mm^3. CD4 count exceeded 200/mm^3 in only two patients. Of six patients beginning dialysis in 1995, five have died of AIDS. Of 10 patients beginning dialysis in 1996, five have died of AIDS.

We conclude that HIVAN is a late complication of HIV-1 infection. Most seropositive patients with ESRD have an AIDS-defining condition before initiating dialysis. Because the high mortality rates for seropositive patients receiving hemodialysis are caused by the presence of AIDS, improved overall survival may only be possible if aggressive antiretroviral therapy is begun before the onset of ESRD.

Pulmonary Diseases

Patterns of Asthma Admissions to a Metropolitan Hospital: 1990–1995. A. Jaramillo, G. Haralambou, N. Rienzi, and E. N. Schachter. Pulmonary Division, Department of Medicine, Mount Sinai School of Medicine, New York, NY.

Recent epidemiologic surveys suggest that the prevalence, morbidity, and mortality of asthma continues to increase in large metropolitan areas. There is concern that these increases are more pronounced in indigent populations and that socioeconomic factors may be partially responsible. We examined asthma-initiated admissions to the emergency room of Mount Sinai Medical Center, a 1071 bed hospital in upper Manhattan, from January 1, 1990 to December 31, 1995. Data were obtained through the Medical Records Department and included age, sex, race, and ethnicity, payment system, and length of stay (LOS). There were 1156, 1233, 1196, 1391, 1191, and 1188 admissions in each year from 1990 to 1995, suggesting a plateauing of this parameter of morbidity. Adult admissions generally increased in the years subsequent to 1990, whereas pediatric admissions increased until 1993 and then declined. During the study period, the composition of admissions by race and ethnicity remained constant with nearly 30% of the admissions being African American and 60% being Hispanic. In general, coverage by payor system also remained stable with most patients covered by Medicaid. In 1995, 61 admissions received health maintenance organization coverage compared with none in 1990. The mean LOS among pediatric patients increased from 3.53 ± 1.95 to 3.88 ± 2.23 days ($p < 0.01$), whereas the mean LOS for adults decreased from 7.31 ± 16.62 to 5.62 ± 5.34 days ($p < 0.05$). There was little difference in LOS among different financial care provider systems for both adults and pediatric patients.

In conclusion, these data from a large urban medical center show that asthma morbidity as reflected by admission frequency and LOS continue to show high prevalences in minority/lower socioeconomic status groups. The data suggest a plateauing of asthma morbidity in this community during the past 5 years.

Antigen Trafficking in Airway Epithelial Cells. T. Kalb, E. Salik, M. Tyorkin, and K. Sperber. Division of Pulmonary/Critical Care and Clinical Immunology, Department of Medicine, Mount Sinai School of Medicine, New York, NY.

In animal models, lower airway instillation of ovalbumin (OVA) induces an asthma-like syndrome and nasal challenge may elicit a specific immune response in distant mucosal sites. The cells that participate in antigen presentation in the airway are characterized incompletely, but may include resident dendritic cells and macrophages. In addition, airway epithelial cells (AEC) share properties with these classic antigen-presenting cells, including class II molecule expression and stimulation of allogeneic T lymphocytes. In the present study we investigated whether AEC are capable of soluble antigen uptake and intracellular trafficking. AEC isolated from surgical specimens (nasal turbinates and segmental bronchi) and human airway cell lines derived from the upper airway (BEAS-2b) and lower airway (A549) were maintained in culture with or without the addition of interferon-γ (IFN-γ) or granulocyte-macrophage colony-stimulating factor (GM-CSF) for 48 hours before the addition of fluorescein-labeled soluble antigen [keyhole limpet hemocyanin (KLH) or OVA]. Intracellular punctate staining by both antigens was detected by fluorescent confocal microscopy, peaking at 60 minutes and persisting up to 180 minutes in all cell types. IFN-γ and GM-CSF pretreatment resulted in more brisk and intense uptake, peaking at 30 minutes, similar to alveolar macrophages. Intracellular trafficking of KLH was confirmed by colocalization with antibody markers for acidic vesicle (dAMP), early endosomal (cathepsin D), late endosomal (M6PR), and lysosomal compartments (CD63).

In conclusion, upper and lower airway-derived epithelial cells show similar capacity for uptake of soluble antigens and that this property is enhanced by inflammatory cytokines. We speculate that AEC may participate in the airway mucosal immune response to inhaled antigen.

Comparative Yield from Three Sputum Specimens for the Diagnosis of Pulmonary Tuberculosis in Patients with AIDS and in a Control Group. S. Manawar, M. S. Ahmad, and M. A. Khan. Pulmonary Division, Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

To determine the maximum number of sputum specimens necessary to confirm the diagnosis of pulmonary tuberculosis (TB), we retrospectively studied 56 patients with culture-positive pulmonary TB. All patients underwent sputum induction. Three specimens were obtained in each case. Forty-six (82%) patients (30 males, 16 females; age range, 13–69 years) were HIV-negative, whereas 10 (18%) patients (9 males, 1 female; age range, 34–51 years) had AIDS. Among 46 HIV-negative patients, 33 (72%) were smear-positive whereas 13 (28%) were smear-negative. Of 33 smear-positive patients, 30 (91%) had all three sputa positive on culture; 1 (3%) showed a positive culture only from the second specimen, whereas 2 (6%) showed a positive culture only from the third specimen. Of 13 smear-negative patients, 6 (46%) were culture-positive from the first specimen, 6 (46%) were culture-positive from the second specimen, whereas only one (8%) was culture-positive from the third specimen. Among the 10 patients with AIDS, 6 (60%) were smear-positive whereas 4 (40%) were smear-negative. Of the 6 smear-positive patients with AIDS, 2 (33%) were culture-positive from all three specimens, 1 (17%) from the first and second specimens, and 3 (50%) from the first specimen only. Of four smear-negative patients with AIDS, 1 (25%) was culture-positive from the first two specimens, 2 (50%) were culture-positive from the first specimen only, whereas one (25%) was culture-positive from the second specimen only. Comparison between the patients with AIDs and without AIDS showed that 6 (60%) of 10 patients with AIDS were smear-positive compared with 33 (72%) of 46 patients without AIDS ($p > 0.124$). Data pooled together for 56 patients showed that 45 (80%) received a diagnosis via the very first sputum specimen, 8 (14%) only by the second specimen, and 3 (6%) only by the third specimen. Most patients with pulmonary TB receive a diagnosis by the very first sputum specimen, whereas the second and the third specimens contribute a small yet significant number of cases. Three sputum specimens remain essential for the diagnosis of pulmonary TB.

Complications of Bronchoscopy in Patients with AIDS. S. Manawar, M. S. Ahmad, and M. A. Khan. Pulmonary Division, Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

Fiberoptic bronchoscopy is considered a relatively benign procedure. In this study, we evaluated whether this procedure results in a higher complication rate in patients with AIDS. We prospectively followed up patients with AIDS after a diagnostic bronchoscopy for 1 week. Evaluation included the appearance of fever, leukocytosis, hemoptysis, pneumothorax, or a new infiltrate on chest x-ray. Forty-one patients with AIDS (30 men, 11 women; mean age, 45.2; age range, 24–67 years) underwent bronchoscopy from July 1995 to September 1996 in our department. The procedure was performed under topical spray anesthesia using 10 cc of 2% lidocaine solution. No patient required conscious sedation. All patients underwent bronchoalveolar lavage, whereas seven patients underwent transbronchial lung biopsy as well. Only one patient (2.4%) developed fever, leukocytosis, and a new infiltrate; sputum culture revealed *Pseudomas aeruginosa* which was treated successfully. There were no other complications. We conclude that the rate of complications after bronchoscopy in AIDS patients is low.

Prevalence of Drug-Resistant Tuberculosis in an Inner-City Community: A Comparison Between Patients with AIDS and a Control Group During Two Consecutive 3-Year Periods. S. Manawar, M. S. Ahmad, and M. A. Khan. Pulmonary Division, Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

To assess the magnitude of drug-resistant tuberculosis (TB) in patients with AIDS in Paterson, NJ, we retrospectively reviewed clinical records of all patients with AIDS who have TB and a control group from January 1991 through December 1993 and from January 1994 through September 1996. The first group comprised 60 patients with AIDS matched with 60 control subjects. Seven (12%) patients with AIDS had drug resistance compared with 4 (7%) control subjects. The second group consisted of 29 patients with AIDS, matched with 29 control subjects. Three (10.4%) patients with AIDS had drug resistance compared with 3 (10.4%) control subjects. Pooled together, there were 14 patients with drug-resistant TB in the second group of whom 10 (71%) were foreign-born. These results indicate that drug-resistant TB both among the patients with AIDS and the general population in this community did not show a significant increase ($p = 0.11$), whereas the foreign-born patients continued to represent a disproportionately higher prevalence of drug resistance.

Rate of Tuberculin Skin Test (PPD) Conversion Among Close Contacts of Patients with Active Pulmonary Tuberculosis. S. Manawar, M. S. Ahmad, and M. A. Khan. Pulmonary Division, Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

This prospective study deals with the results of an epidemiologic surveillance of 31 patients with active pulmonary tuberculosis. Investigation of the close contacts of these patients uncovered 135 persons with an initial negative purified protein derivative (PPD) (<5 mm). The tuberculin skin test was repeated 3 months later when 11 (8.14%) contacts were found to have converted to a positive PPD (≥5 mm). There was no significant difference in the conversion rates between the household group (conversion rate, 7.14%) and the nonhousehold group (conversion rate, 9.8%) of close contacts ($p = 0.823$). Age-based analysis of the tuberculin converters, showed that, of 40 contacts <16 years of age, 2 (5%) converted whereas, of 95 contacts ≥16 years of age, 9 (9.5%) converted to a positive PPD ($p = 0.528$). These results indicate comparable tuberculin-conversion rates between the two age groups during the 3-month observation period. We therefore conclude that the current recommendations of the Advisory Committee for the Elimination of Tuberculosis to provide preventive therapy to tuberculin-negative contacts among the children and the adolescents during the 3-month observation period should be expanded to include the tuberculin-negative adult contacts as well.

Prevalence of Hemoptysis in Community-Acquired Pneumonia. S. Manawar, M. S.. Ahmad, S. Lodaya, and M. A. Khan. Pulmonary Division, Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

Hemoptysis as a symptom of community-acquired pneumonia has been reported rarely. In this study, we evaluated the prevalence of hemoptysis in the patients with community-acquired pneumonia. We prospectively observed 40 patients (23 men, 17 women; mean age, 53.9 years; age range, 27–92 years) with community-acquired pneumonia who were admitted to our hospital and subsequently followed up for the symptoms of cough, sputum production, fever, pleuritic chest pain, and hemoptysis. All patients had consolidations on their chest roentgenograms without cavitation. All patients were treated with antibiotics and showed clinical improvement, accompanied with radiologic improvement in consolidation. Six patients (15%) had hemoptysis. The quantity of blood was ≥5 cc in all patients. There were no other pulmonary diseases to explain the symptom of hemoptysis except pneumonia. All six patients underwent fiberoptic bronchoscopy. No endobronchial malignancy was found in any patient. We conclude that hemoptysis in community-acquired pneumonia is neither unusual nor infrequent.

A Comparison of the Diagnostic Yields Between Transbronchial Lung Biopsy and Bronchoalveolar Lavage in Patients with *Pneumocystis carinii* Pneumonia. S. Manawar, S. Lodaya, M. S. Ahmad, and M. A. Khan. Pulmonary Division, Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

To compare the diagnostic yield between transbronchial lung biopsy (TBLB) and bronchoalveolar lavage (BAL) in patients with AIDS with *Pneumocystis carinii* pneumonia (PCP) we retrospectively reviewed the medical records of patients with AIDS who underwent fiberoptic bronchoscopy for the diagnosis of PCP. Only those patients who underwent both TBLB and BAL were selected for analysis. Seven patients (4 women, 3 men; age range, 21–50 years; mean age, 35 years) received a diagnosis of PCP on TBLB. Only four of these seven (57%) received a diagnosis of PCP from BAL. A comparison between the diagnostic yields of TBLB and BAL performed concomitantly showed that TBLB is superior to BAL in confirming the diagnosis of PCP. BAL alone would have missed the diagnosis of PCP in three (43%) of our patients. Negative BAL does not exclude PCP patients with AIDS, whereas a negative TBLB virtually excludes this diagnosis. We recommend that TBLB must be obtained in patients with AIDS suspected to have PCP when no contraindications to a biopsy exist. Concomitant BAL does not appear to enhance the diagnostic yield of PCP in this setting.

Optimal Criteria for the Diagnosis of Exudative Pleural Effusions. S. Manawar, S. Lodaya, and M. A. Khan. Pulmonary Division, Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

Several criteria have been proposed for the diagnosis of exudative pleural effusions. To select the most sensitive and specific criterion, we prospectively studied 23 pleural effusions applying the following criteria: 1) pleural fluid/serum protein ratio >0.5; 2) pleural fluid/serum lactate dehydrogenase (LDH) ratio >0.6; 3) pleural fluid LDH >200 IU/L; 4) pleural fluid cholesterol >45 mg/dL. Thirty pleural fluid specimens from 23 patients (15 women, 8 men; age range, 21–80 years) were analyzed for protein, LDH, and cholesterol content. Serum samples were obtained concomitantly, and serum proteins and serum LDH were measured. Applying the exudative criteria 1 through 3 listed above, 21 of 30 pleural effusions could be categorized as exudative, whereas 22 of 30 pleural effusions were categorized as exudative when using the cholesterol criterion. Specificity for all exudative criteria except cholesterol was 100%, and sensitivity for all exudative criteria except cholesterol was 95.6%. Using the cholesterol criterion, however, the specificity and sensitivity for exudative effusion was 100%. One pleural effusion categorized as an exudate under cholesterol criterion and as a transudate under all other criteria, occurred in a patient with malignant pleural effusion confirmed on autopsy. Of all the criteria listed above, only cholesterol (>45 mg/dL) criterion of exudative effusion correctly diagnosed all exudates. Using the cholesterol criterion as a sole diagnostic test for exudative pleural effusions is both specific as well as sensitive, besides being extremely cost-effective, because it obviates the need for measuring protein and LDH in the pleural fluid and the serum.

Activity of the Branched Chain Amino Acid-Preferring Component of the Multicatalytic Proteinase Complex (Proteasome) Mirrors Expression of the Inducible Subunit, LMP7. J. Nelson, C.

Altschuller, A. Loukissa, and C. Cardozo.[1] Departments of Medicine and Pharmacology, [1]Division of Pulmonary Diseases, Mount Sinai School of Medicine, New York, NY.

The multicatalytic proteinase complex (MPC) is a very large, extralysosomal proteolytic particle that is essential for eukaryotic cell survival. Among five distinct MPC proteolytic activities, the branched-chain amino acid-preferring (BrAAP) component of MPC may be particularly important in processing immunogenic peptides, which typically have a branched-chain amino acid at the C-terminus. Our previous studies showed that in selected organs such as lung and spleen, which are exposed to heavy antigenic challenge, the BrAAP component is markedly activated, in association with incorporation of three interferon (IFN)-γ-inducible MPC subunits, including LMP7. In these studies, we asked whether BrAAP proteolytic activity correlated with expression of inducible subunits in MPC from native human cells stimulated by various humoral mediators. Third-passage human umbilical vein endothelial cells (HUVEC) were cultured for 72 hours with either medium alone or medium containing IFNγ tumor necrosis factor-α (TNFα), interleukin (IL)-1β, or endotoxin (lipopolysaccharide; LPS), in physiologically relevant concentrations. BrAAP proteolytic activity was increased 2- to 3-fold in IFNγ-treated HUVEC when measured in cell supernatants and in purified MPC. Lesser changes in BrAAP were seen after exposure to TNFα, whereas IL-1β and LPS had no significant effect on the BrAAP activity of MPC in cell supernatants. Changes in BrAAP activity were correlated with LMP7 levels. We conclude that both subunit content (LMP7) and proteolytic function (BrAAP activity) of MPC in primary cultures of human cells may be influenced by the cytokine milieu. Our studies provide further evidence of linkage between activation of the BrAAP proteolytic component and incorporation of cytokine-inducible subunits into the MPC. Cytokine stimulation of inducible subunits and BrAAP proteolytic activity may improve efficiency of antigen processing and thereby serve to adapt the MPC to the unique and specific immunologic challenges of diverse tissues and organs.

Vitamin D Deficiency Is Prevalent in Chronically, Critically Ill Patients. D. M. Nierman and J. J. Mechanick. Divisions of Pulmonary/Critical Care and Endocrinology/Metabolism, Mount Sinai School of Medicine, New York, NY.

The "chronically critically ill" are patients who have survived a critical illness but remain profoundly debilitated and ventilator dependent. We evaluated the prevalence of calcium homeostasis disorders in these patients that might impede their recovery from critical illness.

Methods: Between March and August 1997, 55 consecutive ventilator-dependent patients who transferred to the Respiratory Care Unit (RCU) from one of the Intensive Care Units (ICU) were evaluated. Within 48 hours of RCU admission, serum levels of 25-hydroxyvitamin D_3, 1,25-dihydroxyvitamin D_3, and phenylthiohydantoin (PTH), and a 24-hour urine collection for calcium and N-telopeptide levels were measured.

Results: Forty-one (75%) of the 55 patients had complete data. There were 25 men and 16 women, average age 72 ± 14 years old. Patients were hospitalized an average of 33 ± 24 days before RCU admission, and all had received enteral and/or parenteral nutritional support during their ICU stay. Twenty (49%) patients came from the MICU, 12 (29%) from the CSICU, 5 (12%) from the NSICU, and 4 (10%) from the SICU. Nineteen (46%) patients required ICU care for medical reasons and 22 (54%) for surgical reasons. Thirty-nine (95%) of the 41 patients were vitamin D deficient; 25 (64%) had low 25-hydroxyvitamin D_3 levels (because of either inadequate intake, lack of sunlight, or impaired hepatic conversion), and the other 14 (36%) had normal 25-vitamin D_3 levels and low 1,25-dihydroxyvitamin D_3 levels (because impaired renal conversion). In addition to vitamin D deficiency, five patients (13%) had resorptive hypercalciuria.

Conclusions: Despite universal nutritional support in ICUs, almost all chronically critically ill patients are vitamin D deficient. We recommend that all of these patients receive 1,25-dihydroxyvitamin D_3 with close monitoring as part of their standard therapy.

Pharmacologic Characterization of Organic Dusts from the Latex Industry. E. N. Schachter, E. Zuskin, N. Rienzi, S. Goswami, S. Maayani, J. Strongwater, and H. Shim. Pulmonary Division, Department of Medicine, Mount Sinai School of Medicine, New York, NY.

In a recent study we evaluated workers exposed to latex dust in the paper recycling industry. Water-soluble extracts from dusts collected from two different processing areas in the recycling plant (LD1 and LD2), were prepared as a 1:10 w/v solution. Dose-related contractions of nonsensitized, guinea pig trachea (GPT) were demonstrated using these extracts. PD1 and PD2 were added to the GPTs in 0.5 log dose increments. Response was measured as a percent of maximal carbachol response. The effects of mediator-modifying drugs, endopeptidase inhibitors, calcium-blocking agents, and capsaicin were tested (see Table 1). Atropine (A) inhibited both PD1 and 2. Pyrilamine (P), indomethacin (I), acivicin (AC), NDGA (N), and TMB8 (T) had variable effects on LD-induced constriction. Capsaicin (C) depleted irritant nerves of bronchoconstricting mediators, but paradoxically, pretreatment with capsaicin produced, in some cases, mild enhancement of LD contractile responses. We conclude that LD extracts cause dose-related contractions of GPT and that these extracts are inhibited by a wide variety of pharmacologic agents.

TABLE 1

E_{max} *(as a percent of control) for LD1 and 2 after pretreatment with pharmacologic agents*

	A	P	I	AC	N	T	C
LD1	11*	63*	58*	58**	65*	84*	100
LD2	3*	70	95	32**	44*	55*	147

*$p < 0.05$; **$p < 0.01$.

Community-Acquired Pneumonia: Determination of the Optimal Duration of Therapy. R. E. Siegel, M. Alicea, A. Lee, and R. Blaiklock. Bronx VA Medical Center, NY; and Division of Pulmonary and Critical Care Medicine, Mount Sinai School of Medicine, New York, NY.

Purpose: Patients hospitalized for treatment of community-acquired pneumonia (CAP) are usually treated with i.v. antibiotics, changed to oral therapy, and discharged. Optimal duration of therapy for CAP is unknown.

Methods: A prospective, randomized, double-blind placebo controlled study of hospitalized patients with CAP was begun to determine if 7 days of therapy is sufficient. Patients were assigned to one of two treatment groups and followed up for 6 weeks. Both groups were treated with 2 days of i.v. cefuroxime (Zinacef®) 750 mg q8h for six doses. Then patients received oral cefuroxime axetil (Ceftin®) 500 mg q12h for either 8 days or 5 days (plus 3 days of placebo).

Results: Interim analysis after 42 patients entered into the study: 37 patients evaluable (18 in group 1 and 19 in group 2). Data for evaluable patients include all male; mean age, 61.4 years; 51% Black; 30% Hispanic, and 19% White. No differences were noted between groups in age, comorbidities, smoking, lobes involved, pleural effusion, positive blood cultures, O_2 saturation, creatinine, admission temperature, or white blood cell count. Cure rate was similar, 89% in group 1 and 83% in group 2 (95% C.I. for a difference of 0.061 is −0.159 to +0.282). All failures were early (<5 days on therapy) and no late recurrences were seen. Resolution of chest film was similar.

Conclusion: Seven days of treatment is adequate therapy for most patients with moderately severe CAP.

Clinical Implications: More than 7 days of therapy for CAP does not improve cure rate but increases the cost of treatment.

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Rheumatology

Hidradenitis Suppurativa Complicated by Erosive Arthropathy and Peripheral Ulcerative Keratitis. N. Abid, A. Opran, and Z. Kamal. Department of Internal Medicine and Rheumatology, Queens Hospital Medical Center, NY.

Background: Hidradenitis suppurativa (HS) is a disease of the apocrine glands of the skin. Our patient with this disorder developed recurrent peripheral ulcerative keratitis (PUK) of Mooren's ulcer type and erosive arthritis of the wrists.

Case Report: A 44-year-old African American man with a history of HS with extensive skin involvement had a severe exacerbation of the skin condition. At that time, he developed pain, redness, and decreased vision in his left eye. He had a 360° peripheral corneal ulcer with 90% thinning and undermined edge, and significant conjunctival congestion. In addition to his multiple suppurative skin lesions, he had club-bing of the fingers, but without joint abnormalities. He had a normochromic normocytic anemia, polyclonal-hypergammaglobulinemia, and a positive rheumatoid factor (titer of 1:321). The patient was treated with oral prednisone (80 mg daily), and left eye conjunctival resection with marked improvement. Two years later, the patient's skin condition flared again and he developed a painful, red right eye as well as pain and swelling of both wrists. The right eye had PUK of Mooren's type, similar to the findings in the left eye 2 years before. Roentgenograph of the hands showed bony erosions of the ulnostyloid process bilaterally, and bony erosions along the radial aspect of the bases of the second and third proximal phalanges. Oral prednisone therapy (80 mg daily) was restarted, and the patient improved. One year later, the patient developed recurrent Mooren's ulcer of the left eye. Oral cyclophosphamide (100 mg daily) was given in addition to prednisone (80 mg daily). The patient's eye condition improved significantly, as did the skin lesions. One year later, the patient is taking oral cyclophosphamide (50 mg daily) and is free of skin, eye, or joint symptoms.

Discussion: HS is a chronic suppurative and cicatricial disease, primarily involving the apocrine glands in the skin. Musculoskeletal involvement is rare, and usually associated with severe skin flares. Mooren's ulcer is a rare but characteristic form of PUK that progresses circumferentially and centrally. The central margin of the ulcer has a characteristic overhanging lip (undermining edge). The pathogenesis of Mooren's ulcer is unknown, but a localized autoimmune disorder is hypothesized.

Synergistic Effect of IgG on Binding of IgA Autoantibodies to β_2-Glycoprotein I. V. A. DeBari and M. W. Galazka. Division of Rheumatology, Department of medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

Some patients with antiphospholipid syndrome have IgA class antibodies to β-glycoprotein I (β_2GPI). Herein we describe our studies on the effects of endogenous and exogenous IgG autoantibodies to β_2GPI on IgA autoantibody binding. To determine whether endogenous IgG alters the binding of IgG anti-β_2GPI, we exhaustively adsorbed (with recombinant protein G bound to Sepharose, GammaBind G-S) IgG from two serum specimens containing IgG anti-β_2GPI and examined the difference in the titration curves of IgG anti-β_2GPI. To study the effect of exogenous IgG, we preincubated IgG purified by affinity chromatography on GammaBind G-S, with β_2GPI adsorbed on microtiter plates at varying IgG concentrations and a fixed dilution of test specimens. In the former group of experiments (endogenous IgG), we observed a statistically significant difference ($p < 0.01$) in the slopes and intercepts of the titration curves; those with IgG demonstrating increased binding. Preincubation with exogenous IgG resulted in curves which were multiphasic and suggestive of both inhibition and enhancement of IgA binding. These data are consistent with the concept that aggregation plays a role in the exposure of the normally cryptic autoepitope of β_2GPI.

Standardized Immunoassay of IgA and IgM Autoantibodies to β_2-Glycoprotein I in Patients with Antiphospholipid Syndrome. V. A. DeBari, M. W. Galazka, and L. B. Keil. Division of Rheumatology, Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ.

The antiphospholipid syndrome (APS) is a hypercoagulable state which may occur secondary to another autoimmune disease or as a primary clinical entity. Patients with APS exhibit *in vitro* anticoagulant activity related to autoantibodies reactive with β_2-glycoprotein I (β_2GPI) bound to anionic phospholipids or other surfaces capable of presenting a neoepitope, encrypted in the native form of β_2GPI. In this study, we examined the binding of β_2GPI by IgA and IgM from from serum or plasma from 20 patients with APS, all of whom had circulating IgG anti-β_2GPI. Specimens (1:100 dilutions) were subjected to an enzyme immunoassay (EIA) using a microtiter well format. The EIA was a typical "antibody assay" with antigen (β_2GPI) bound to irradiated polystyrene. The probes were peroxidase-conjugated anti-human IgA and IgM. Reference calibrators were used to standardize the assay for standard IgA anti-β_2GPI units (SAU) and IgM anti-β_2GPI units (SMU). Reference intervals (RI) were constructed by determining the 95th percentile of values obtained for a group of 203 healthy adults. These yielded RI of 0–7 SAU and 0–13 SMU. APS patients had 1–195 (range) SAU (mean, 35 SAU) with 17 of 20 patients above the upper limit (UL) of the RI; IgM ranged from 4 to 206 SMU in the APS patients (mean, 61 SMU), with 16 of 20 patients above the UL of the RI. IgA and IgM anti-β_2GPI correlated well with β_2GPI-dependent anticardiolipin antibodies (IgA: $r = 0.82$, $p < 0.0001$; IgM: $r = 0.84$, $p < 0.0001$). IgG anti-β_2GPI previously has been shown to be a useful marker for the diagnosis of APS. The addition of IgA and IgM completes a panel of major immunoglobulin class autoantibodies which may further enhance our diagnostic capabilities.

A Stable Multisubunit Complex of β_2-Glycoprotein I. V. A. DeBari, M. Galazka, S. Kelly, J. Li, J. Kohles, L. B. Keil, and M. Petersheim. Division of Rheumatology, Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ: and Department of Chemistry, Seton Hall University, South Orange, NJ.

The demonstration that an epitope on β_2-glycoprotein I (β_2GPI) is the target for autoantibodies from patients with antiphospholipid syndrome has sparked interest in this hitherto obscure protein. β_2GPI is a phospholipid-binding protein which resembles the complement family of proteins in that it is composed of a series of five short consensus repeats or "sushi" domains. It is also unusual in that it has a high carbohydrate content with N-linked glycans terminating in sialic acid residues accounting for nearly 25% of the molecular weight of the protein. During the course of our studies on β_2GPI, we subjected the protein to polyacrylamide gel electrophoresis (PAGE) both with sodium dodecyl sulfate (SDS-PAGE) and without SDS (native PAGE) and to atomic force microscopy (AFM) imaging of the protein adsorbed to molybdenum sulfide (MoS_2). SDS-PAGE (under reducing conditions) of β_2GPI from three sources indicates that the 54 kDa β_2GPI band is accompanied by a band corresponding to an 8-kDa protein. In the absence of detergent and reducing agents (native-PAGE), β_2GPI demonstrates a complex which is dissociable by boiling in 6–8 M urea, yielding several lower molecular mass bands, one of which corresponds to the 8-kDa protein observed in SDS-PAGE. AFM images of native β_2GPI show aggregates of up to 20 particles, each having a diameter of 30–35 nm. This is consistent with a globular unit the size of which would be substantially larger than that expected for a 54-kDa protein. These experiments suggest that the 54-kDa β_2GPI monomer subunits exist as a multimeric complex with the 8-kDa protein.

Presentation of Cryptic Autoepitope of β-Glycoprotein I by Treatment with Glutardialdehyde. V. A. DeBari, M. Galazka, J. Kohles, M. Tang, L. B. Keil, and M. Petersheim. Division of Rheumatology, Department of Medicine, St. Joseph's Hospital and Medical Center, Paterson, NJ and Department of Chemistry, Seton Hall University, South Orange, NJ.

Autoantibodies from patients with antiphospholipid syndrome (APS) recognize an epitope on β_2-glycoprotein I (β_2GPI) only when native β_2GPI id adsorbed on surfaces composed of anionic phospholipids or oxidized polystyrene. We modified β_2GPI with the cross-linking agent, glutardialdehyde (GDA), which induced exposure of the anti-β_2GPI epitope at GDA:β_2GPI mole ratios in the range of 500 to 2000. A second cross-linking agent, dimethylsuberimidate (DMS), does not expose the epitope which may be a consequence of its having less tendency than GDA to form intermolecular links. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis experiments demonstrate that GDA does promote extensive intermolecular cross-linking of β_2GPI and DMS does not. Formaldehyde also reacts with the lysine residues of β_2GPI, but does not expose the epitope. The circular dichroism (CD) spectra of native and modified β_2GPI were obtained. Changes in the amplitude of the composite CD band at 235 nm confirm that GDA induces changes in conformation which are qualitatively different from those caused by formaldehyde. These data provide further evidence for the role of the lysine residues in controlling exposure of the autoepitope. The results also support the possibility that anti-β_2GPI antibodies may bind to aggregates of the protein and that circulating β_2GPI might be subject to alterations which lead to loss of tolerance and induction of autoepitopes under appropriate conditions.

The Under-Diagnosis of Rheumatic and Musculoskeletal Problems During Hospitalization for General Medical Admission: Implications for Training and Patient Care in Internal Medicine and Rheumatology. M. Karlekar, A. Lef, M. H. Ornstein, L. D. Karr, and H. Spiera. Department of Medicine, Mount Sinai School of Medicine, New York, NY.

Objective: Musculoskeletal and rheumatic complaints account for numerous outpatient visits to internists. With the current trend toward primary care training, internists will have to recognize and treat many basic musculoskeletal problems now seen by specialists. The purpose of this study was to determine the prevalence of undiagnosed rheumatic complaints in an inpatient general medical ward of a university teaching hospital.

Methods: Sixty-five patients were selected randomly from two different medicine wards, 48 hours after admission. The intern and resident notes and orders were reviewed carefully with respect to the musculoskeletal and rheumatic history, current rheumatic complaints, and physical findings. The patients were evaluated next by a resident physician and attending rheumatologist participating in the study. Patients were categorized as having a Class I rheumatic condition if a major rheumatic complaint or finding was found by the study team that was not recognized by the admitting physicians and would have a significant impact on the patient's normal daily activities. Class II rheumatic conditions were categorized by minor complaints or findings that were not recognized by the admitting physicians, that would have a minimal impact on daily activities, but were recognizable entities.

Results: Sixty-five patients completed the study; 38 of 65 patients were found to have either a Class I or II rheumatic condition (58.5%, 95% CL = 48%), 20 of 65 patients were found fulfilling a Class I category (30.8%, 95% CL = 21.4%); 18 of 65 patients had a Class II rheumatic condition (30%, 95% CL = 21%). Subanalyses revealed commonly missed conditions.

Conclusions: Common rheumatic conditions are frequently underdiagnosed by residents training in Internal Medicine during hospitalization of patients on a general medical ward. At times the unrecognized conditions were serious and interfered with the patient's daily activities. Further efforts are needed to mandate formal training of medical students and residents on the importance of recognizing and treating rheumatic conditions.

Sudden Hearing Loss in Patients with Systemic Lupus Erythematosus or Lupus-Like Syndromes and Antiphospholipid Antibodies: A Report of Five Patients and a Review of the Literature. M. Naarendorp and H. Spiera. Division of Rheumatology, Department of Medicine, Mount Sinai School of Medicine, New York, NY.

Although the pathogenesis of sudden hearing loss in patients with systemic lupus erythematosus (SLE) is not clear, several reports suggest an association with the antiphospholipid antibody (APA). We report five patients with SLE or a lupus-like syndrome, who had sudden hearing loss and had elevated levels of APA or the lupus anticoagulant. A review of the medical literature revealed an additional five cases. Of these five additional cases two patients were not tested for APA. The remaining three had elevated APA levels. These data suggest that the sudden hearing loss in patients with SLE, who have elevated levels of APA, is a manifestation of the antiphospholipid syndrome. As such, we recommend that these patients be treated with heparin, followed by high-intensity warfarin (producing an INR of 3.0 or greater), with or without low-dose aspirin.

Microvascular Thrombi Mimicking Palmar Vasculitis in Systemic Lupus Erythematosus Associated with Anticardiolipin Antibodies: A Report of Three Cases and Review of the Literature. M. H. Ornstein, R. G. Phelps, and F. Swerdlow. Division of Rheumatology, Department of Medicine and Department of Dermatopathology. Mount Sinai School of Medicine, New York, NY.

The antiphospholipid syndrome (APS) has been associated with macroscopic thrombotic events, and more recently, episodes of clotting in the microvasculature. Previously described cutaneous manifestations associated with antiphospholipid (aPL) antibodies have included: livedo reticularis, cutaneous ulcerations, diffuse cutaneous necrosis, and pyoderma gangrenosum. Cutaneous nodules and macules have been documented in two previous case reports, in three instances stimulating vasculitis. Palmar vasculitis consisting of scattered maculopapular and nodular lesions is a common occurrence in patients with systemic lupus erythematosus (SLE). We now describe three patients with SLE associated with the presence of aPLs, who presented with palmar skin lesions simulating classic cutaneous vasculitis. Skin biopsies documented small vessel fibrin thrombi, without the presence of vasculitis. Although corticosteroid therapy was relatively ineffective; treatment with anticoagulation resolved the lesions, without sequelae. We suggest that the possibility of microvascular thrombosis should be considered in any patient with SLE who presents with palmar skin lesions resembling vasculitis. This current report supports the observation that thrombosis in the microvasculature is an important component of the APS.

Spinal Cord Research

Subjects with Chronic Cervical Spinal Cord Injury and Hyperresponsive to Ultrasonically Nebulized Distilled Water. E. Arias, D. R. Grimm, M. Lesser, W. A. Bauman, and P. L. Almenoff. Spinal Cord Damage Research Center, Bronx VA Medical Center, NY; and Departments of Medicine and Rehabilitation Medicine Services, Mount Sinai School of Medicine, New York, NY.

Individuals with chronic cervical spinal cord injury (SCI) demonstrate bronchial hyperreactivity in response to aerosolized methacholine and histamine, pharmacologic agents that act directly on specific receptors on airway smooth muscle. It has not been determined whether these subjects also respond to nonspecific provocative agents. Therefore, to determine whether subjects with chronic cervical SCI are also hyperresponsive to a physicochemical agent, 15 subjects (9 with tetraplegia and 6 with paraplegia), including smokers and nonsmokers, were challenged with ultrasonically nebulized distilled water (UNDW; Ultra neb 99, DeVilbiss Co., Somerset, PA; particle size, 0.5–5 microns; output, 3.0 mL/min). After determination of baseline spirometric parameters, subjects were challenged with normal saline, followed by UNDW for progressively increasing periods of time (30 seconds, 1, 2, 4, and 8 minutes). Spirometry was performed immediately and 2 minutes after each challenge. The PD_{20} was defined as the provocative dose of distilled water (mL) causing a 20% decrease in forced expiratory volume in 1 second (FEV_1), and was calculated using a computer program which generated the value by interpolation from a logarithmic dose-response curve. For those subjects not responding to the maximal concentration of distilled water (24 mL/min), a PD_{20} value of 24 mL was used in the calculation of mean PD_{20}. To UNDW challenge, 8 or 9 subjects with tetraplegia had a significant response (mean ± SD, $PD_{20} = 9.97 = 7.67$ mL), whereas 0 of 6 subjects with paraplegia responded ($PD_{20} > 24$ mL). These data are consistent with previous studies that demonstrated a mean PD_{20} of 9 mL in asthmatic subjects and >24 mL in healthy subjects (Thorax 1983; 38:284). The observation that subjects with chronic cervical SCI are hyperresponsive to an osmotic stimulus adds further support to the hypothesis that airway hyperreactivity in these individuals is caused by end-organ, nonspecific bronchial hyperresponsiveness.

Ethnic and Gender Differences in the Serum Lipid Profile in Individuals with Chronic Immobilization Compared with Able-Bodied Controls. W. A. Bauman, A. M. Spungen, R. Herbert, C. Schecter, and D. Smith. Divisions of Endocrinology and Cardiology, Department of Community Medicine, Mount Sinai School of Medicine, New York, NY; and Spinal Cord Damage Research Center and Rehabilitation Medicine Services, Bronx VA Medical Center, Bronx, NY.

Persons with spinal cord injury (SCI) have premature cardiovascular disease. In the general population, a serum lipid profile of depressed serum high-density lipoprotein (HDL) cholesterol level is associated with increased risk for coronary heart disease. Several reports have demonstrated that the primary lipid abnormality in the population with SCI is a depressed serum HDL cholesterol value. From 1993 to 1996, the serum lipid profiles were investigated in 320 subjects with SCI from Rancho Los Amigos Medical Center, CA, and compared with those obtained in 303 Bridge and Tunnel Officers (BTOs), New York. Serum lipid profiles were performed on all subjects after a minimum of 4 hours fasting. All laboratory analyses from New York (BTOs) and California (subjects with SCI) were performed by the same clinical laboratory facility (Quest Diagnostics, Inc., Teterboro, NJ). All serum lipid profile values were significantly different between the two groups. The serum HDL cholesterol level was reduced in the SCI compared with the BTO group (mean ± SEM) (42 ± 0.79 vs 47 ± 0.67 mg/dL, $p < 0.0005$). The serum HDL cholesterol was inversely related to both body mass index (BMI) (SCI: $r = 0.27$, $p < 0.0001$; BTOs: $r = 0.22$, $p < 0.0001$) and estimated percent body fat (SCI: $r = 0.27, p < 0.0001$; BTO: $r = 0.22$, $p < 0.0001$) in both groups. Serum HDL cholesterol values were approximately 8 mg/dL lower for any given BMI in those with SCI. The slope of serum HDL cholesterol with estimated percent fat in those with SCI was significantly steeper than in the BTO group (−0.546 ± 111 vs −0.246 ± 0.062, $F = 5.9$, $p = 0.015$). The serum HDL cholesterol values were inversely correlated with serum triglycerides in both groups (SCI: $r = 0.42$, $p < 0.0001$; BTOs: $r = 0.43$, $p < 0.0001$). The serum HDL cholesterol level was significantly different for males between the SCI and BTO groups (39 ± 0.83 vs 45 ± 0.70 mg/dL, $p < 0.0001$), but not for females (51 ± 1.54 vs. 54 ± 1.52 mg/dL, NS). Within the SCI and the BTO groups, African Americans had higher serum

HDL cholesterol values than Whites or Latinos (SCI: 49 ± 1.56 vs 41 ± 1.02 or 37 ± 1.53 mg/dL, $p < 0.0001$, respectively; BTOs: 51 ± 1.27 vs 46 ± 0.86 mg/dL, $p < 0.01$ or 42 ± 1.59 mg/dL, $p < 0.0005$, respectively). Serum HDL cholesterol values remained lower in subjects with SCI than in BTOs between subgroups for Whites and Lations (Whites: 41 ± 1.02 vs 46 ± 0.86 mg/dL, $p < 0.0001$; Latinos: 37 ± 1.53 vs 42 ± 1.59 mg/dL, $p < 0.05$), but not for African Americans (49 ± 1.56 vs 51 ± 1.27 mg/dL, NS). Thus, immobilization from SCI imparts an unfavorable serum lipid profile in White and Latino males, but generally not in females or African Americans.

Extreme Inactivity Does Not Affect Serum Lipoprotein (a) Levels. W. A. Bauman, A. M. Spungen, R. Herbert, C. Schecter, and D. Smith. Divisions of Endocrinology and Cardiology, Department of Community Medicine, Mount Sinai School of Medicine, New York, NY; and Spinal Cord Damage Research Center and Rehabilitation Medicine Services, Bronx VA Medical Center, Bronx, NY.

The apoprotein moiety of lipoprotein (a) [Lp(a)] bears a striking similarity in structure, but not in function, to plasminogen. Serum Lp(a) has been hypothesized to impart potential thrombogenicity when levels are elevated. The lipid component is very similar to low-density lipoprotein (LDL) cholesterol. A growing body of evidence suggests that elevated levels of serum Lp(a) confer increased risk of vascular disease. Contradictory findings have been reported for the effect of exercise on Lp(a). Some investigators have demonstrated that exercise increases Lp(a), whereas others have found no change. To determine the effect of activity on the concentrations of Lp(a), another possible approach is to investigate levels in persons who are at the lowest end to the activity spectrum. Serum levels of Lp(a) have not been reported in persons who are paralyzed. The level of serum Lp(a) in a ethnically mixed population of patients with chronic spinal cord injury (SCI) was compared with a similar ethnically diverse population of able-bodied persons. The distribution and correlates of serum Lp(a) levels were studied in 524 subjects followed up as out-patients who were being seen for routine annual physical examinations at the Comarr Spinal Injury Clinic at Rancho Los Amigos Medical Center, Downey, CA, and in a group of 388 relatively sedentary, able-bodied subjects who were Bridge and Tunnel Officers of the Metropolitan Transportation Authority employed by the state of New York. Serum Lp(a) for both groups was performed by Quest Diagnostics, Inc., Teterboro, NJ. A significant difference was not found for serum Lp(a) levels between the groups (19.5 ± 0.75 vs 19.9 ± 1.00 mg/dL). These data had the power to detect a 20% difference between the SCI and BTO groups of 0.90, and to detect a 30% difference, 0.99. Neither age nor duration of SCI had an effect on serum Lp(a) levels. Ethnicity had a significant effect on serum Lp(a) levels in both groups. In those with SCI, serum Lp(a) levels were significantly higher in the African American group than in the Latino or White groups [28 ± 1.7 vs 16 ± 0.9 and 18 ± 1.3 mg/dL ($F_{(2)} = 27.7, p < 0.0001$)] and in the able-bodied subjects [33 ± 2.3 vs 15 ± 2.0 and 15 ± 1.0 mg/dL ($F_{(2)} = 38.6, p < 0.0001$)]. When comparing within each ethnic group for significant differences between those with SCI and those without, no significant difference was found.

Prolonged immobilization does not appear to have a significant effect on serum Lp(a) levels compared with ambulatory individuals. The well-described ethnic differences in serum Lp(a) levels in the able-bodied population were demonstrated as well in persons with severe immobilization.

Bronchoconstriction in Subjects with Cervical Spinal Cord Injury is Not Reversed by Lung Inflation. D. R. Grimm, R. V. De Luca, M. Lesser, W. A. Bauman, and P. L. Almenoff. Spinal Cord Damage Research Center, Bronx VA Medical Center, Bronx, NY; and Departments of medicine and Rehabilitation Medicine Services, Mount Sinai School of Medicine, New York, NY.

Bronchial hyperreactivity to both methacholine (Mch) and histamine (Hist) has been observed consistently in subjects with cervical spinal cord injury (SCI). This hyperresponsiveness has been attributed to the loss of sympathetic airway innervation resulting in unopposed parasympathetic (bronchoconstrictor) activity. Recent evidence suggests that the bronchial hyperresponsiveness may also be caused by the decreased lung volumes in SCI and the resulting loss of the normal airway smooth muscle stretch mechanism that occurs with deep inspiration. We tested the ability of lung inflation to reverse Mch ($n = 4$)- or Hist ($n = 9$)-induced bronchoconstriction in subjects with cervical SCI. Subjects inhaled Mch or Hist in increasing concentrations until a 20% decrease in FEV_1 from baseline occurred (PC_{20}). All 13 patients had a $PC_{20} < 8$ mg/mL, which is considered positive in our laboratory. The mean ± SD, PC_{20} value was 1.43 ± 1.47 mg/mL for Mch and 1.92 ± 1.30 mg/mL for Hist groups. The mean % change in forced expiratory volume in 1 second (FEV_1) for the Mch group was −24% and for the Hist group −40%. A volume cycled ventilator (Lifecare PLV-102) with a mouthpiece attached was then applied to administer eight tidal breaths at a volume between 60 and 70% of forced vital capacity (FVC). After lung inflation, the mean % change in FEV_1 was −25.5% for the Mch group and −34.7% for the Hist group. There was no statistical difference between the change in FEV_1 after bronchial challenge and lung inflation. These data reveal that lung inflation does not reverse induced bronchoconstriction in subjects with cervical SCI. The findings suggest that airway smooth muscle in subjects with cervical SCI fails to stretch normally with deep inhalation, possibly because of microatelectasis associated with chronic restrictive ventilatory defect.

Calcium Ion Blockers Normalized Thrombin-Induced Release of Platelet-Derived Growth Factor from Platelets in Spinal Cord Injury. N. Kahn, W. Bauman, and A. Sinha. Division of Spinal Cord Research, Mount Sinai School of Medicine, New York, NY; and Spinal Cord Damage Research Center, Bronx VA Medical Center, Bronx, NY.

Coronary artery disease (CAD) is accelerated in subjects with chronic spinal cord injury (SCI) and the underlying mechanism is poorly understood. Because increased platelet aggregation (PA) stimulates atherosclerosis, and inhibition of PA by prostacyclin (PGI_2) may retard atherogenesis, we determined the role of PGI_2 in platelet function in SCI. Previously, we had shown that the SCI platelets were not hypersensitive to aggregating agents nor resistant to the inhibitory effect of PGI_2 to PA. However, SCI platelets did not control the platelet-stimulated thrombin generation and the release of platelet-derived growth factor (PDGF). Because PDGF, a mitogen, is known for the development of atherosclerosis, we investigated the release of PDGF by thrombin in the circulation of SCI subjects ($n = 12$) compared with nonSCI subjects ($n = 12$). PDGF levels were assayed by an enzyme-linked immunosorbent assay (ELISA) system. The basal plasma PDGF level in SCI subjects was three times higher (6.41 ± 0.12 ng/mL) than controls (2.15 ± 0.12 ng/mL); the thrombin-induced release of PDGF was also consistently higher in SCI (24.2 ± 1.2 pg/10^6 cells) when compared with nonSCI (15.11 ± 1.6 pg/10^6 cells. Pretreatment of ScI platelets with 100 nM prostaglandin E_1 (PGE_1) (used as a stable probe of prostacyclin) inhibited the release of the mitogen by 90% ($p < 0.001$) in control platelets. Similar treatment of SCI platelets had no such affect. Scatchard plot showed that the inability of PGE_1 to reduce PDGF release to normal may be caused by the loss of high-affinity prostacyclin receptors ($Kd_1 = 9.1 \pm 2.0$ nM vs $Kd_1 = 7.2 \pm 1.1$ nM) on the platelet surface. Pretreatment of SCI platelets with 10 mM verapamil, a calcium ion channel blocker, "corrected" the release of PDGF to "normal" ranges ($p < 0.001$). (High-affinity sites after verapamil treatment have not been studied). These results suggest that the use of a calcium ion channel blocker, such as verapamil, might be beneficial in those with SCI by "normalizing" the release of PDGF.

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VOLUME 65 NUMBER 2 MARCH 1998

MAR -2 1998

CONTENTS

THEME ISSUE

THE RENIN-ANGIOTENSIN SYSTEM AND TARGET ORGAN DAMAGE IN HYPERTENSION

Clive Rosendorff and Steven A. Atlas, guest editors

Introduction: The Renin-Angiotensin System—from Tigerstedt to Goldblatt to ACE Inhibition and Beyond *Steven A. Atlas and Clive Rosendorff* 81

The Renin-Angiotensin System Revisited: Classical and Nonclassical Pathways of Angiotensin Formation *Steven A. Atlas* 87

Left Ventricular Hypertrophy in Hypertension: Etiology, Treatment, and Controversies *Robert A. Phillips, Joseph A. Diamond, and Ali Gharavi* 97

Angiotensin II and Cardiac Remodeling *Willa A. Hsueh, Yung S. Do, and Raju Jeyaseelan* 104

Vascular Hypertrophy in Hypertension: Role of the Renin-Angiotensin System *Clive Rosendorff* 108

The Renin-Angiotensin System as a Mediator of Renal Injury in Hypertension *Ali Gharavi and Paul Klotman* 118

Preventing and Reversing Target Organ Damage by Treatment of the Renin-Angiotensin System *Lawrence R. Krakoff and Vinutha Rajasekharaiah* 125

DEAN'S SEMINAR

Antagonizing Excitotoxicity: A Therapeutic Strategy for Stroke? *Dennis Choi* 133

continued inside

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Veterans Affairs

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THE
MOUNT SINAI JOURNAL
OF MEDICINE

Volume 65
Number 2
March 1998

CONTENTS *continued*

ORIGINAL ARTICLES

Immunoglobulin and IgG Subclass Levels in the African American and Hispanic Populations of East Harlem *Moshe Lewis, Michelle Miyashiro, Jennifer Huton, Lorraine Miller, and Kirk Sperber* 139

Amaranthin Lectin Binding in the Rat Colon: Response to Dietary Manipulation *Evren O. Atillasoy, Anargyros Kapetanakis, Steven H. Itzkowitz, and Peter R. Holt* 146

COMMENCEMENT ADDRESS

In Sickness and in Health *Christine K. Cassel* 154
Mount Sinai School of Medicine, May 16, 1997

DERMATOLOGY NOTES

Reactive Perforating Collagenosis *Mark Lebwohl and Stephen Kurtin* 158

Required Form for Authors .... *inside back cover*

Introduction

The Renin-Angiotensin System—From Tigerstedt to Goldblatt to ACE Inhibition and Beyond

Steven A. Atlas, M.D., and Clive Rosendorff, M.D., Ph.D.

Renin, the protease that catalyzes the rate-limiting step in the formation of the pressor peptide angiotensin II, was discovered nearly a century ago (1). Only during the past decade or so, however, has the significance of the renin-angiotensin system in the pathogenesis of cardiovascular disorders gained wide acceptance or appreciation. As recently as 20 years ago, when the present authors were just entering this field, many experts were skeptical that angiotensin had much significance in cardiovascular disease beyond its involvement in the development of renovascular and malignant hypertension or in the rare cases of hypertension caused by renin-secreting neoplasms (2, 3).

The recent excitement about renin and angiotensin II as therapeutic targets in cardiovascular disease, although based on extensive basic and clinical investigation during the past 60 years, has been spurred on mainly by the development of angiotensin-converting enzyme inhibitors and other agents that block the system, and also, to some extent, by newer insights gained with the tools of modern cellular and molecular biology. The series of articles that follows will review various aspects of these newer developments. This article provides additional background information for readers who are unfamiliar with the field.

Historical Perspective

In 1898, the Swedish physiologist Robert Tigerstedt and his student Per Bergmann published an account of their experiments which demonstrated a heat-labile substance in crude extracts of rabbit kidney cortex capable of inducing a long-lasting increase in arterial pressure (1). They postulated that the kidney secreted a humoral pressor agent, for which they proposed the term "renin." This concept was disputed by subsequent investigators who were either unable to confirm their work or unable to produce renal hypertension experimentally. The finding was largely ignored until 1934 when Goldblatt and his colleagues (4) published their classical studies showing that renal ischemia produced by clamping of the renal artery led to sustained hypertension. Goldblatt's model fit well with earlier evidence linking renal arteriolar damage to malignant hypertension in humans (5), thereby sparking renewed interest in renin. During the next few years, it was shown that the ischemic kidney released not only a renin-like substance but also a heat-stable, short-lived pressor substance, leading to the discovery that renin was not directly pressor but rather acted enzymatically on a plasma substrate (eventually to be known as "angiotensinogen") to liberate a pressor substance (6, 7). In subsequent attempts to purify this pressor substance (initially termed "angiotonin" or "hypertensin" by competing investigators, who eventually compromised on the term "angiotensin"), two forms of angiotensin (8) were discovered. This led to the recognition that a second

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Key Words: renin, angiotensin, angiotensinogen, angiotensin-converting enzyme, aldosterone, hypertension, sodium balance

enzyme, "angiotensin-converting enzyme," was required to convert an inactive precursor (angiotensin I) to the active humoral agent (angiotensin II). Angiotensin II was purified, sequenced, and synthesized (9–12), and by the late 1950s, synthetic peptide was made available for physiological experiments.

Angiotensin II was among the most powerful direct arteriolar vasoconstrictor agents known at the time (12). Nonetheless, using the crude assay methods then available, investigators were unable to demonstrate an association between either renin or angiotensin levels and normal blood pressure control or experimental or clinical hypertension, leaving the significance of this hormonal system in doubt. An additional, unanticipated major action was discovered, however. Aldosterone, the principal mineralocorticoid hormone produced by the adrenal cortex, was discovered during the same period that the chemical nature of angiotensin II was being revealed (13). A series of indirect but intriguing findings at the same time clearly established a close interrelationship between renal and adrenocortical function and the control of extracellular fluid volume (14–16). These observations led several groups of investigators to expose angiotensin II as a major regulator of aldosterone secretion (17–20). Thus, by 1960, our current concept of the renin-angiotensin-aldosterone system and its role in the homeostatic control of blood pressure and extracellular volume had been outlined in broad terms.

Control of Angiotensin II Formation

The operation of the renin-angiotensin system is considered in greater detail in the article by Atlas in this issue (see p. 87). Its classical formulation as a circulating endocrine system can be summarized as follows (see ref. 21 for review). The principal regulatory step is the control of renin secretion by the kidney, where renin is synthesized and stored in modified myoepithelial cells (the juxtaglomerular cells) that usually predominate along the afferent arteriole. Release of renin is stimulated by a decrease in renal perfusion, by sodium depletion, and by sympathetic (β_1-adrenergic) stimulation. The effect of decreased perfusion is mediated by activation of the stretch-sensitive juxtaglomerular cells and by a decrease in sodium and/or chloride ion delivery to the distal tubule, which is sensed by a cluster of specialized distal tubular epithelial cells (the macula densa) that lie in close apposition to the juxtaglomerular cells. On release, renin catalyzes the generation of the decapeptide angiotensin I from angiotensinogen, a glycoprotein of hepatic origin, and angiotensin I in turn is converted to the octapeptide angiotensin II by the action of angiotensin-converting enzyme, an ectoenzyme that lines most vascular endothelium but is particularly abundant in the pulmonary microcirculation. Angiotensin II, via its vasoconstrictive, aldosterone-stimulating and other related effects (see below), restores or increases arterial pressure and extracellular volume. These actions serve to defend the circulation when increased renin secretion is provoked appropriately by systemic signals such as volume contraction, upright posture, or hypotension. Restoration of volume and renal perfusion usually will turn off the signals that increase renin secretion, but these homeostatic responses may be impaired in pathological states such as heart failure, cirrhosis with ascites, and related edematous disorders, in which elevated angiotensin levels contribute to pathological fluid accumulation while failing to fully restore "effective" arterial volume. Also, when increased renin secretion is inappropriate to the needs of the systemic circulation (e.g., with selective underperfusion of the kidneys caused by renal artery stenosis), the increased levels of renin and angiotensin II will lead to hypertension.

The foregoing formulation of the renin-angiotensin system has been broadened considerably during the past decade to include the possibility of local regulation of angiotensin II formation at the tissue or even cellular level, along with the concept of autocrine and/or paracrine actions of the peptide (22). These issues, and the controversies surrounding them, will be addressed in this series of articles.

Actions of Angiotensin II

Angiotensin II is the major effector hormone of the system, at least in the classical view. [The potentially important, and possibly unique, actions that have been ascribed to other angiotensin metabolites will be considered in a subsequent article.] As already implied, most of the actions of angiotensin II that have been described to date (23) work together to defend the circulation in the face of volume loss or other causes of hypotension. Aside from direct vascular smooth muscle constriction, effects that potentially can contribute to the support of arterial tone include facilitation of norepinephrine release at sympathetic nerve terminals, catecholamine release by the adrenal medulla, and stimulation of sympathetic outflow by a central effect. Effects on vascular endothelium, including stimulation of endothelin release and inhibition of nitric oxide and vasodilator prostaglandin release, may also modulate vascular tone and tissue perfusion. In addition to sympathetic facilitation, evidence suggests that angiotensin may modulate cardiac output by a direct inotropic effect on the heart. Volume conservation is achieved not only by increased aldosterone production, but also by a direct effect of angiotensin II to promote proximal tubular sodium reabsorption and by central effects to increase thirst and stimulate antidiuretic hormone synthesis and release. In addition, its effects on intrarenal vascular and mesangial contractility may affect glomerular perfusion and function; in particular, angiotensin II seems to be an important controller of efferent arteriolar tone, and consequently of glomerular filtration pressure. Aside from these acute actions, angiotensin has growth-promoting effects that may contribute to vascular smooth muscle hyperplasia and to the processes of cardiac hypertrophy and remodeling, both via myocyte hypertrophy and stimulation of fibroblast proliferation and extracellular matrix production. These actions are considered in detail in other articles in this series. Angiotensin can also promote increased glucocorticoid production, inhibit prolactin release, and stimulate hepatic glycogenolysis, although the significance of these actions is not understood as well.

Virtually all these known actions are mediated by a single class of cell surface receptor, the AT_1 receptor, which belongs to the superfamily of G-protein-linked receptors with seven membrane-spanning regions (24). As discussed elsewhere in this issue, activation of this receptor leads to increases in cytosolic calcium via activation of phospholipase C and the generation of inositol trisphosphate and diacylglycerol. The function of a second major receptor class (AT_2) is unknown, but AT_2 seems to be more abundant during early development and may mediate antigrowth effects; its relevance to physiological or pathological processes has not yet been elucidated.

The Development of ACE Inhibitors

As indicated above, the discovery and development of inhibitors of angiotensin-converting enzyme (ACE) has played a major role in the relatively recent appreciation of the significance of angiotensin in cardiovascular morbidity and mortality. Before that, two other approaches already had indicated that blockade of the renin-angiotensin system was likely to be beneficial in the treatment of hypertension. First, the antihypertensive action of propranolol, and subsequently other beta-blockers, was shown to be related largely to its ability to inhibit renin secretion (25). Second, a peptide analogue of angiotensin II, saralasin (sar^1, ala^8-angiotensin II), was shown to be a receptor antagonist (26), which also lowered blood pressure acutely (27, 28). The usefulness of this agent was limited, however, because it was not orally active and because it was a partial receptor agonist, which thus underestimated the degree to which blood pressure might be elevated by angiotensin II (28).

The first compounds used as ACE inhibitors, similarly limited to intravenous use, were a series of peptides extracted from the venom of the South American pit viper (*Bothrops jararaca*). These peptides had been identified a decade earlier as factors that potentiated the actions of bradykinin (29), and it was only after the discovery that ACE was identical with one of the kinin-degrading enzymes (kininase II) that these peptides were shown to inhibit ACE as well (30). One of these, the nonapeptide teprotide, was synthesized by scientists at the Squibb Institute and made available to investigators. The experience with teprotide in experimental and clinical hypertension (3, 31–33) provided the first convincing evidence that angiotensin II contributed to the maintenance of elevated blood pressure in a significant proportion of patients with hypertension and provided a major impetus for the development of an orally active compound. Through a series of remarkable insights and deductive reasoning, scientists at Squibb designed a highly effective oral agent, captopril (34), which was introduced for clinical trials in 1977 (3, 35, 36).

During the ensuing decade, this compound and a series of second- and third-generation ACE inhibitors became mainstays in the treatment of cardiovascular disorders. Although their antihypertensive efficacy is clearly greater in clinical situations in which the renin-angiotensin system is activated, their action is sufficiently broad-based that, combined with an excellent side-effect profile, they have become widely used as first- or second-line agents in the treatment of essential hypertension (37). Shortly after their introduction, ACE inhibitors were shown to have potent cardiac unloading effects (38), and they have since proven to be invaluable for the treatment of heart failure, leading to often dramatic symptomatic improvement and increased survival (39, 40). Beyond this, the ACE inhibitors have shown promising therapeutic value in situations that were not clearly anticipated at the time of their development, such as renal protection, cardiac hypertrophy and remodeling, and survival after myocardial infarction (41–44). The latter results have been all the more surprising because the reductions in morbidity and mortality clearly extend to clinical subsets in which the renin-angiotensin system is not overtly activated. These issues will be addressed in subsequent articles in this issue.

Renin, Angiotensin, and Cardiovascular Risk

As implied earlier, the clinical benefits of ACE inhibition documented recently have suggested a broad role for angiotensin II as a pathogenetic factor in cardiovascular disease. This concept has not yet gained universal or unequivocal acceptance, however, because ACE inhibitors are not specific for the renin-angiotensin system in that they also block the degradation of kinins and other potentially relevant peptides. The tools to resolve this issue are now available with the recent development of highly specific, orally active blockers of the system, namely renin inhibitors (which have not yet been made clinically available) and specific competitive antagonists of both the AT_1 and AT_2 receptor subtypes (24). A new therapeutic chapter is unfolding as well. AT_1 receptor antagonists recently have been introduced for the treatment of hypertension (45); although experience to date indicates a similarity to the ACE inhibitors in terms of the spectrum of action, the potential clearly exists for significant differences to emerge with further study.

Beyond the initial results of therapeutic interventions, numerous experimental findings and epidemiological observations have supported this broad role for angiotensin II. First, for a long time angiotensin has been thought to contribute directly to vascular damage, particularly in the setting of hypertension or volume overload (46), and numerous growth-promoting and related proatherogenic and prothrombotic effects have been identified in recent years (23, 47). Second, plasma renin levels among patients with hypertension have been linked to the risk of myocardial infarction and stroke, initially by retrospective analyses (48, 49). More recent prospective studies have confirmed plasma renin levels as an independent risk factor for heart attack (but not stroke) in patients with hypertension rivalling even cholesterol and other acknowledged risk factors in their predictive value (50, 51).

A third and equally compelling line of evidence is emerging from the study of gene polymorphisms of renin, angiotensinogen, ACE, and the AT_1 receptor in humans. Previous epidemiological findings suggested that plasma angiotensinogen levels are related to blood pressure (52). Two relatively common variants of angiotensinogen, missense mutations leading to the substitution of threonine for methionine at codon 235 (M235T) and the substitution of methionine for threonine at codon 174 (T174M), have been associated with modest increases in the prevalence of essential hypertension in some populations (53, 54); although there are also negative reports regarding these two mutations, linkage studies at least suggest a close association of the angiotensinogen locus to hypertension (55). The 235T allele, which is associated with slightly higher plasma levels of angiotensinogen, also appears to be a risk factor for development of pre-eclampsia (56) and may predispose to cardiac hypertrophy (57). Even more persuasive are the findings with a polymorphism in the ACE gene caused by the variable insertion (I) or deletion (D) of a noncoding 287-base-pair sequence in intron 16. The D allele has been associated with increased risk of myocardial infarction, particularly in otherwise low-risk individuals (58); although it is still controversial, this finding has been confirmed in several study populations (59, 60). In addition, initial reports suggest an association of the DD genotype with increased risk of dilated cardiomyopathy, coronary restenosis, cardiac and vascular hypertrophy, and possibly even diabetic nephropathy (61–65). The I/D polymorphism accounts for roughly half the variance in plasma ACE levels; the D allele is associated with higher levels. Although plasma ACE is not thought to be instrumental in the conversion of angiotensin I to II, some studies indicate that tissue ACE levels are also increased in subjects with the ID and DD genotypes, and it has been proposed that the I/D polymorphism may be linked to another as-yet-unidentified mutation which alters ACE gene transcription rates (60). As will be explored in another article in this series, it is possible that an alteration in ACE gene expression may lead to enhanced local production of angiotensin II at a tissue level and thereby contribute to vascular damage independently of hypertension per se. Beyond this, preliminary evidence shows that the association of the DD genotype with increased risk of myocardial infarction may be enhanced significantly by the coexistence of an independent mutation in the AT_1 receptor gene because of a base transversion (adenine to cytosine) at nucleotide position 1166 (66). These intriguing findings from population genetics, combined with the impetus from clinical and experimental observations, have opened a new era of scientific inquiry into the mechanisms whereby the renin-angiotensin system, beyond its documented influences on blood pressure and volume control, contributes so profoundly to cardiovascular and renal disease.

Conclusion

Work performed during the past century has revealed the renin-angiotensin system as a multifunctional system, with angiotensin II, the main effector, regulating many physiological functions, including vascular tone, sodium and volume homeostasis, and blood pressure. In addition, angiotensin II has been shown to stimulate growth of vascular smooth muscle, cardiac myocytes, and a variety of renal and other cell types. These findings, combined with evidence from epidemiological studies linking acquired or genetically determined dysregulation of the renin-angiotensin system to cardiovascular risk, suggest that angiotensin II may play an important role in the development of cardiovascular and renal diseases associated with vascular and myocardial hypertrophy, including not only hypertension and heart failure, but also atherosclerosis, restenosis after angioplasty, the ventricular remodeling associated with myocardial ischemia, and diabetic nephropathy. The articles that follow have been chosen and structured to provide insight into some of these major target organ effects, with emphasis on the role of the renin-angiotensin system, and the potential for the control of these effects by ACE inhibitors, by AT_1-receptor antagonists, and in the future, possibly by other pharmacologic or gene-based interventions which will act on the renin-angiotensin system.

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The Renin-Angiotensin System Revisited: Classical and Nonclassical Pathways of Angiotensin Formation

Steven A. Atlas, M.D.

The renin-angiotensin system is an unusual endocrine axis in which the active hormone, angiotensin II, is formed in the extracellular space by sequential proteolytic cleavage of its precursors, a pathway that is initiated by the regulated secretion of renin, the rate-limiting processing enzyme. Mediated by the direct actions of angiotensin II on vascular smooth muscle, the kidney, the central nervous system, and the adrenocortical production of aldosterone, this system is involved in the homeostatic control of arterial pressure, tissue perfusion, and extracellular volume (1).

This system has been viewed traditionally as a systemically acting circulating endocrine cascade in which angiotensin II is formed in the blood and from there delivered to its various target organs. In evaluating alterations in this system in various physiological and pathological states, much of the emphasis has been placed on measurement of plasma-renin levels. This has occurred because the secretion of renin by the kidney is the principal focus of endocrine regulation in this cascade, and also for historical reasons related to methodological considerations. This approach reinforced the notion of a systemic-endocrine cascade.

In recent years, however, increasing emphasis has been placed on the concept of "local" or "tissue" angiotensin production. This arose from two principal lines of evidence. First, it has been shown that several tissues are able to express the genes for renin or other components of the system. Second, there is the discovery that additional proteolytic enzymes are able to synthesize angiotensin peptides, at least *in vitro*. Furthermore, this concept is reinforced by clinical and experimental observations that suggest a beneficial response to pharmacological agents that block the renin-angiotensin system even under circumstances in which the circulating system is not activated appreciably (2–5). This article will consider the significance of these more recent concepts after reviewing the classical pathway of angiotensin II production.

Regulation of Angiotensin Biosynthesis: The Classical View

The synthesis of angiotensin is initiated by the proteolytic action of renin on angiotensinogen, a circulating globulin of hepatic origin. Renin (EC 3.4.23.15) is an aspartyl (acid) protease which is synthesized at low levels by several extra-renal tissues and is produced abundantly by the juxtaglomerular cells of the kidney (1, 6). Like most secreted proteins, renin is synthesized as a precursor (prorenin) and stored in secretory granules. It is unusual in that intracellular processing to the active form is incomplete, so that considerable amounts of prorenin are stored and cosecreted with renin. In the circulation, prorenin is

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the dominant form in some species, accounting for 70% to 90% of the total immunoreactive renin in humans (7). There is no evidence that prorenin is converted to renin in the blood, and the functional significance of this finding is unknown. However, the presence of prorenin in the blood has important implications for the accurate measurement of renin because it cross-reacts with many antirenin antibodies and is easily converted to active renin under unphysiological circumstances *in vitro* (7, 8).

The juxtaglomerular cells, which are modified myoepithelioid cells that line mainly the afferent arteriole of the glomerulus, are the principal (if not the exclusive) source of active renin secreted into the circulation (1, 9). Renin secretion, which is the principal regulatory step in the renin-angiotensin cascade, is stimulated by two intrarenal mechanisms that are activated by a decrease in renal perfusion pressure. The first mechanism is a renal baroreceptor signal caused by stretch sensitivity of the juxtaglomerular cells; the second is a decrease in delivery of sodium chloride to the distal nephron which is sensed by a cluster of specialized tubular epithelia (the macula densa) that closely apposes the juxtaglomerular cells of the afferent arteriole. Renin secretion is also regulated systemically via changes in renal sympathetic nerve traffic stimulated by β_1-adrenergic activity (1).

Acting in the extracellular space (including blood), renin cleaves the N-terminal decapeptide of angiotensinogen, forming the biologically inactive precursor, angiotensin I. Angiotensinogen is an α_2-globulin whose gene exhibits a high degree of structural and organizational homology with the serpin (serine protease inhibitor) gene superfamily (10). It has presumably lost (or failed to acquire) protease inhibitory activity to become the unique substrate for renin during its evolution. The renin cleavage site is a Leu^{10}-Val^{11} bond in human angiotensinogen, but Leu^{10}-Leu^{11} in most other species (11, 12). Angiotensinogen is secreted constitutively by the liver (although it is also synthesized at low levels by certain other tissues), and its circulating levels usually remain fairly constant in an individual. Its synthesis is induced by estrogens and, to a lesser extent, by glucocorticoids, thyroid hormone, and angiotensin II (13). Because its concentration in the blood and extracellular fluid (in most species) is usually in the range of its K_m for renin, the generation of angiotensin I is a first-order process with regard to angiotensinogen as well as to renin. Angiotensinogen levels do not vary over the short term, i.e., minutes to hours. Most day-to-day variations in angiotensin formation rates depend on changes in renin secretion. Because the active hormone end-product (angiotensin II) has a direct negative feedback effect on renin secretion (1, 14), it is possible that a resetting of renin secretion may even compensate for long-term variations in angiotensinogen levels. Such compensation is probably incomplete, however. For example, raising angiotensinogen concentrations by infusing the pure protein into sodium-depleted rats can raise blood pressure (15), presumably by leading to sustained increases in angiotensin II.

The conversion of angiotensin I to the active octapeptide hormone angiotensin II occurs by removal of the C-terminal dipeptide by a zinc metalloprotease known as angiotensin-converting enzyme (ACE) (EC 3.4.15.1), which acts as a dipeptidyl carboxypeptidase, cleaving angiotensin I between residues Phe^8-His^9 (16). Angiotensin I is not the only substrate for this enzyme, which also degrades bradykinin and a number of other peptides, but the conversion of angiotensin I to II is thought to be its major catalytic function *in vivo.* Although ACE is present in blood, the formation of angiotensin II is thought to be catalyzed mainly by the membrane-bound form found abundantly in vascular endothelium, in which ACE is present as an ectoenzyme embedded in the cell membrane with its catalytic sites (two per molecule) exposed to the extracellular space (17). Because of the particularly high density of ACE in the pulmonary microcirculation, the lung is probably a major site of angiotensin II formation (17, 18). ACE is also found in other cell types, notably in the brush border of absorptive epithelia, such as in the renal proximal tubule. The conversion of angiotensin I to angiotensin II is not considered rate limiting under normal physiological conditions, because the reaction rate depends mainly on the concentration of angiotensin I. ACE levels are thought to be fairly constant. Glucocorticoids and thyroid hormone have been reported to increase ACE production *in vitro.* Theoretically, if the density of membrane-bound ACE is altered under physiological or pathological conditions it is possible that the rate of angiotensin II production (as discussed below), may be influenced. Again, this influence depends on the degree to which angiotensin II can induce compensatory feedback inhibition of renin secretion.

In the classical view of the renin-angiotensin system as a circulating endocrine system, increased production of angiotensin II is initiated by an increase in renin secretion, which is stimulated under physiological conditions by a decrease in renal perfusion or sympathetic activation caused by upright posture, hypotension, and sodium depletion. As described above, the rise in plasma renin then provides the rate-limiting step in an enzymatic cascade leading to the production of the active hormone angiotensin II, which is delivered via the bloodstream to its target organs, e.g., vascular smooth muscle, kidney, and adrenal cortex. In this construct, if one accepts the evidence that the pulmonary microcirculation is the major site of angiotensin I to II conversion (18), this system appears efficiently designed to deliver the active hormone specifically to the systemic arterial circulation.

Angiotensin II acts by binding to specific membrane receptors of the AT_1 subtype (a member of the G-protein coupled receptor superfamily linked to activation of phospholipase C) which are responsible for virtually all the known actions of the peptide (19, 20). The enhanced vascular tone and renal sodium conservation, induced by a direct effect on the kidney and tubule cells and by an indirect effect via increased aldosterone, serve to restore blood pressure, extracellular volume, and renal perfusion, all of which modulate the stimulus for renin secretion. Again, factors that regulate renin in effect serve to regulate angiotensin.

Nonclassical Modes of Angiotensin Generation: General Considerations

The construct of the renin-angiotensin system as a purely endocrine system is considered too narrow by most authorities and has been challenged in recent years with increasing emphasis on the concept of tissue renin-angiotensin systems (21–24). As noted above, a major impetus for this challenge arose because the results of clinical studies and trials show that the beneficial effects of drugs that block the renin-angiotensin system have not always correlated closely with measurements of plasma renin or angiotensin levels. As considered below, there is considerable evidence for the existence of renin or renin-like enzymes in several tissues, but their origin, particularly in vascular tissues and the heart, is controversial.

Unfortunately, the ongoing debate, which has focused mainly on the issue of tissue renin, is confused not only by polemic (25, 26) but also by a major semantic issue. To many, the concept of a "tissue system" automatically implies production of angiotensin II by locally synthesized components. Since the advent of sensitive techniques for measuring messenger RNA (mRNA), it has been possible to detect expression of the genes for renin, angiotensinogen, and ACE in several tissues—including brain, heart, vascular smooth muscle, and adrenal. Although these have been termed tissue "systems," actual demonstration of a functionally intact pathway for entirely local generation of angiotensin II is frequently lacking. In many cases, not all components are expressed by the same tissue or cell type, and little information or clear hypotheses have been presented to explain how these systems might be integrated or regulated. Apart from such tissue renin systems, it has also been proposed that in some tissues proteolytic enzymes other than renin and ACE may be involved in the generation of angiotensins I and II, as well as additional angiotensin metabolites that may have significant independent actions. As will be considered below, much more work is required before these intriguing hypotheses can be either accepted or rejected.

On the other hand, it is plausible that a major fraction of biologically active angiotensin II is generated locally, even if initiated and sustained by renin and angiotensinogen delivered via the bloodstream. In fact, the idea is not new, because angiotensin II was originally classified by pharmacologists as an "autacoid" rather than a hormone. The concept of an endocrine system was strongly influenced by a series of experiments that examined the fate of intravenously infused angiotensin I (18), an experimental paradigm that favors first-pass metabolism by the lung but also one that does not necessarily pertain to the mode of synthesis *in vivo*. Kinetic considerations suggest strongly that the angiotensin peptides, in particular angiotensin II, are likely to be synthesized in close proximity to their target cell, and as stated above, ACE is almost ubiquitous. Moreover, based on the known affinity of angiotensin II for its receptors, as measured *in vitro*, one might predict that the levels of the peptide measured in plasma are frequently too low to have a significant biological effect. An important corollary of the concept of local generation is that regional increases in angiotensin synthesis might occur, e.g., in pathological processes, without systemic feedback of angiotensin II on renin secretion, so that local variations in angiotensinogen or ACE (or even other proteases) might have relevance.

Recent experiments examining the fate of angiotensin peptides that were infused intraarterially provide evidence of significant regional generation of angiotensin II (27–29). Such local synthesis would be of even greater relative importance if angiotensin I were generated locally as well. It is obviously important to determine the extent to which such local generation is driven by renin secreted by the kidney. But beyond that, the ultimate question is whether any locally regulated mechanisms exist which influence the rate of synthesis of angiotensin II.

Extrarenal Renin and Renin Gene Expression

Renin-like enzymatic activity has been detected many extrarenal tissues. As will be discussed in detail below, there are many other tissue proteases which may mimic renin *in vitro* and may not necessarily reflect a physiological angiotensin-generating mechanism. Nonetheless, what appears to be authentic renin has been demonstrated in vascular tissue, heart, brain, pineal, pituitary, uterus, placenta, ovary and adrenal, among others (30–39). However, the degree to which local synthesis of renin contributes to these findings is not yet fully resolved because the tissue levels are often very low, and contamination by, or uptake from, blood may be difficult to exclude. In particular, studies of aorta and other peripheral blood vessels show a rapid decline in tissue levels after bilateral nephrectomy, with virtually complete disappearance by 24 to 48 hours (40–42). Also, infused exogenous renin is readily taken up by vascular tissue (42, 43). These studies raise the intriguing possibility of compartmentalization of the renin reaction, but one that may be driven by the kidney.

Nonetheless, it is almost certain that local synthesis contributes to the renin found in many of the tissues cited above, particularly pineal, placenta, uterus, and adrenal, where levels are too high to be accounted for by contamination by circulating renin (32, 35, 37, 38). Even in vascular tissue, evidence from studies in cultured vascular smooth muscle (44, 45) and endothelial cells (46), which eliminate the issue of blood contamination, suggests the possibility of *in situ* synthesis. However, the possible contribution of nonspecific proteases is difficult to exclude.

Further evidence supporting the potential capacity for a variety of tissues to synthesize renin comes from more recent studies of renin gene expression. There is little disagreement about renin synthesis by adrenal, reproductive organs, and pituitary, in which there is colocalization of renin mRNA and immunoreactive renin (6, 47–49). Furthermore, renin gene expression appears to be regulated in these tissues, as well as in the brain (50), suggesting potential physiological significance. Considerable controversy remains regarding the significance of renin gene expression by cardiac and vascular tissue, however (25, 26). Some investigators have detected renin message in these tissues by Northern hybridization (47, 48), but the specificity of these findings has been questioned (25) and negative results have been reported using perhaps more stringent conditions (51–53). Whereas at least one group has confirmed the presence of renin mRNA in rat cardiac tissue by the solution hybridization technique (48), others have been unable to do so (50). Conflicting findings have even been reported with the most sensitive technique for detecting trace quantities of mRNA, namely polymerase chain reaction (PCR) amplification of reverse-transcribed RNA (cDNA). Negative results have been reported for cardiac renin gene expression in rodents and humans by some workers (49, 53), whereas others have reported the presence of the renin message by pushing this technique to the limits of sensitivity (52, 54, 55). Besides the risk of artifacts with this technique caused by illegitimate transcription, some have questioned the physiological relevance of such low levels of renin gene expression, particularly because it apparently is not under physiological regulation (52). Moreover, it is highly doubtful that the levels of gene expression detected, or the amounts of renin-like activity released by cultured cells, are sufficient to account for the levels of enzymatically active renin detected in whole-tissue extracts. These reservations aside, it is not possible to discount entirely the hypothesis that such low level expression of the renin gene might affect cellular function (26).

Another important issue that must be resolved is the degree to which any locally synthesized renin is active *in vivo.* As mentioned earlier, renin is secreted with prorenin by the kidney (7). Although renin disappears from the circulation after bilateral nephrectomy (9, 40), detectable amounts of prorenin remain in the blood (7, 9, 56). That prorenin is easily susceptible to activation *in vitro* (56) probably accounts for some spurious reports of active renin or angiotensins in anephric human plasma. Moreover, the presence of prorenin, not active renin, in the plasma of humans who have undergone nephrectomy suggests that any extrarenal renin that reaches the circulation may have been released as the inactive precursor. Consistent with that possibility, several studies reporting the existence of renin in extrarenal tissues indicate that it is present considerably, if not exclusively, as prorenin (35, 37, 39, 57–59). The question remains whether any renin activity detected in tissue homogenates or cell culture supernatants may have arisen from *in vitro* (i.e., unphysiological) activation of prorenin.

Tissue Expression of Angiotensinogen and Angiotensin-Converting Enzyme

Far less controversy exists regarding the tissue expression of the other components of the renin-angiotensin system. Besides the liver, the angiotensinogen gene is expressed in the kidney, brain, pituitary, adrenal, reproductive organs, brown adipose tissue, large arteries, and the heart (13, 47, 60), and evidence exists for tissue-specific regulation of angiotensinogen under both physiological and pathological conditions (13, 61–64). Controversy exists regarding the localization of angiotensinogen mRNA within the aortic wall, where it has been attributed variously to vascular smooth muscle, the adventitia, or the surrounding brown fat. In theory, locally synthesized angiotensinogen could provide a source for angiotensin formation either within its cell of origin (see below) or after its release. The extent to which such local synthesis might make an impact on extracellular concentrations of angiotensinogen beyond that delivered via the circulation is difficult to predict. It seems to be significant in the kidney and central nervous system. In the kidney there is strong evidence for compartmentalization of angiotensin biosynthesis (65), which appears to occur in both proximal tubular epithelium—the major renal site of angiotensinogen biosynthesis—and the juxtaglomerular cells, where angiotensinogen is present even though its mRNA cannot be detected (66). Expression of angiotensinogen in the brain is regulated by glucocorticoids and may be the source of the appreciable concentrations of angiotensinogen in the cerebrospinal fluid (67). It seems to be synthesized exclusively by the astroglial cells (68), but is believed to act in a paracrine manner, with the secreted protein taken up by neighboring neurons where it serves as a source for the formation of angiotensin II, which functions in turn as a neuromodulatory peptide.

An even stronger case can be made for the significance of so-called “tissue” ACE expression, at least in the sense that endothelial ACE is widely expressed throughout the microcirculation of every organ, including the vasa vasorum (17, 69, 70). This membrane-bound form of the enzyme is also distributed widely throughout the central nervous system (71) and is expressed by the brush border of the renal proximal tubule and other absorptive epithelia (e.g., choroid plexus, small intestine) as well as other cell types (70, 72). The solubilized form, present in plasma and cerebrospinal fluid, arises by proteolytic cleavage near the C-terminal segment that usually anchors the enzyme to the plasma membrane. A lower molecular weight isoform, which arises from the same gene after activation of a second internal promoter sequence, which contains only a single catalytic center, is expressed in the germinal epithelium of the testes (69, 70, 73, 74). Besides the general regulatory factors mentioned earlier, there is some evidence for possible tissue-specific regulation in pathological states. For example, ACE has been reported to be up-regulated in ventricular hypertrophy caused by pressure overload (75), in experimental heart failure (76), and in the contralateral kidney of experimental renovascular hypertension (77). The mechanisms causing these alterations in ACE levels are not known.

Evidence for Local Angiotensin II Production

As indicated above, ample evidence exists that angiotensin II is synthesized *in situ* in the kidney and central nervous system. Within the kidney, where renin is obviously plentiful and where angiotensinogen is synthesized locally and also delivered via the circulation, compartmentalization of the reaction reportedly shows that interstitial fluid angiotensin II levels are several orders of magnitude greater than those in plasma (65). Similar potential exists in other tissues, e.g., adrenal, pituitary, and ovary, where local synthesis of renin is unequivocal if physiologically regulated. At present, however, the degree to which renin is processed to the active form at these sites is unclear.

Several published reports have provided direct or indirect evidence for local angiotensin production by other isolated tissues, and in particular, vascular tissue and isolated perfused vascular beds (78–84). Limited data also exist for studies in the isolated heart (23, 24). The evidence for generation of angiotensin II in such studies, either by direct detection of the peptide or by its biological activity, is substantial. Many pitfalls exist in the experimental approaches used, however, which prevent reaching firm conclusions regarding physiological relevance. First, tissue autolysis during such experiments might potentially release cellular proteases such as cathepsin D, which are capable of generating angiotensin peptides *in vitro,* particularly when tissues are bathed or perfused by synthetic media which lack naturally occurring protease inhibitors (see below). This is particularly problematic in studies that have used the N-terminal tetradecapeptide of angiotensinogen as a source of substrate for renin (79–81, 83), because this synthetic fragment is hydrolyzed readily by several proteases which are unable to cleave angiotensinogen in physiological conditions. Thus, bioactive angiotensin II could be formed from angiotensin I that has been generated unphysiologically. Second, not all studies controlled for the possibility that angiotensin synthesis was initiated by renin of kidney origin (78, 79). Other studies have used bilaterally nephrectomized animals as a model to study extrarenal renin (82) but, as noted above, a period of 24–48 hours postnephrectomy may not be sufficient to completely clear renin that was taken up from the circulation. Although there may be convincing evidence for local synthesis of angiotensin II where isolated vasculature is concerned, the weight of current evidence favors that it depends largely on renin of kidney origin (85).

Published reports that raise the possibility of functionally significant angiotensin II production by isolated cells. Although this may not be sufficient to account for the amount of peptide detected in whole-tissue extracts, such *in situ* generation of angiotensin II, presumably by locally synthesized components, would fulfill the proposed concept of an autocrine or paracrine function at the cellular level (21). Several cell types, especially those of neural origin, have been reported to express multiple components of the renin-angiotensin system and to synthesize angiotensin peptides (86–89). More recent studies suggest that such cellular synthesis of angiotensin may exert significant effects on cell growth. Antisense oligonucleotides complementary to the angiotensinogen transcription start site have been reported to decrease DNA synthesis by cultured mesangial and neuroblastoma cells (90). In addition, mechanical stretch of cardiac myocytes *in vitro* has been reported to cause release of angiotensin II that in turn appears to initiate a stretch-induced growth response (91). These intriguing observations have yet to be confirmed. Nonetheless, they provide additional support for the concept that angiotensin II can exert an autocrine influence on cellular function.

Alternate Pathways of Angiotensin Biosynthesis

Renin is nearly unique as a circulating active proteolytic enzyme in that it is not inhibited by any of the naturally occurring protease inhibitors in the blood. It has been known for a long time that related acid proteases such as pepsin and cathepsin D ("pseudorenin") are able to cleave angiotensinogen to produce angiotensin I *in vitro* (92), but such proteases do not exist in active form in the blood. More recently, additional proteolytic enzymes have been identified in a variety of tissues that are capable of producing renin-like or ACE-like cleavages of angiotensinogen or angiotensin I, respectively, or even of direct formation of angiotensin II from angiotensinogen. Although such proteases are also unlikely to act in the circulation because of the presence of proteases inhibitors, it is theoretically possible that they could contribute to angiotensin formation at the tissue level.

Both neutral and acid proteases capable of generating angiotensin I from angiotensinogen have been isolated and characterized from a variety of tissues, including brain, uterus, and blood vessel wall (31, 33, 36, 92–94). These renin-like enzymes, variably termed tissue "isorenins" or "angiotensinogenases," have not been identified structurally or ascribed to known proteases. Because they have been isolated from tissue homogenates, it is not known whether they are released into the extracellular space *in vivo,* or if released, whether conditions (e.g., pH) would favor their action. Therefore, the significance of such enzymes in the generation of angiotensin I under either physiological or pathological circumstances must remain speculative.

A better characterized series of enzymes produced *in vitro* a chymotrypsin-like cleavage of angiotensinogen at the Phe^8-His^9 bond, thus directly forming angiotensin II (95–102). These enzymes, which include cathepsin G, elastase, tonin and related kallikrein-like proteases (found in submandibular gland and elsewhere), and tissue plasminogen activator, also are able to exert an ACE-like action by producing the same cleavage in angiotensin I. Cathepsin G and elastase are released by neutrophils after chemotactic stimulation (96–98). Tissue plasminogen activator, whose major action is to stimulate fibrinolysis, is synthesized by vascular endothelium (102). Thus, it has been hypothesized that these proteases might enhance angiotensin II generation locally at sites of inflammation or tissue injury.

In addition to the enzymes mentioned above, other chymotrypsin-like serine proteases related to leukocyte chymases have been isolated from vascular tissues and shown to convert angiotensin I to angiotensin II. Several years ago, it was reported that angiotensin II production by isolated blood vessels from dog, human, and other species was only partially blocked by ACE inhibitors but was blocked completely by the addition of chymostatin (103). This led to the identification of a "chymostatin-sensitive angiotensin generating enzyme" which was localized primarily to the adventitia, not to the predominant endothelial localization of ACE (104). More recently, similar findings using myocardial membrane preparations have led to the identification and purification of a specific chymase-like enzyme from the heart that is clearly distinct from cathepsin G or elastase (105, 106). Unlike the latter proteases or tonin, the purified human heart chymase shows relative specificity for angiotensin I as a substrate (107). It appears that the heart chymase mainly originates from mast cells, but high concentrations reportedly are present in cardiac interstitium. The available data have been widely interpreted as indicating that chymase, not ACE, is the major angiotensin II-forming enzyme in the heart of certain species, including humans. Although this is an intriguing hypothesis with important clinical implications, e.g., regarding ACE inhibitors, it must be remembered that this conclusion is based predominantly on *in vitro* findings. Recent studies using a putative chymase-specific, ACE-resistant, angiotensin I analog suggest the potential existence of a chymase-dependent pathway in both the heart and vasculature of intact animals (108, 109), but further work is needed to determine its relative importance.

Conclusions

The concept of the renin-angiotensin system as a circulating endocrine regulatory pathway has received extensive experimental and clinical validation during the past century. In this construct, the regulated secretion of renin initiates the biosynthesis of angiotensin II, which behaves like a blood-borne hormone that acts on its various distant target organs. Because feedback effects are exerted mainly on renin secretion, the other components of the enzymatic cascade, angiotensinogen and angiotensin-converting enzyme (ACE), have been considered to play a secondary role in the regulation of the system.

The dominance of this view has been challenged extensively, with more recent models emphasizing local or tissue renin-angiotensin systems or on alternate enzymatic pathways of angiotensin II biosynthesis at a tissue level. An abundance of data now supports the possibility of these nonclassical constructs, but current knowledge is fragmentary and somewhat superficial. Validation of the concept of such tissue systems will require more extensive evidence that cellular expression or tissue levels of the various components are clearly subject to physiological regulation and/or dysregulation under pathological conditions. Taken to its extreme, the idea that a single cell, or perhaps cross-talk between two cell types, might be responsible for the synthesis of angiotensin II, which in turn exerts autocrine or paracrine effects, might have been widely discounted just a decade ago. But, seemingly valid models now exist in the central nervous system, the kidney, and perhaps the adrenal, and intriguing findings are beginning to emerge in other tissue types, including the heart and blood vessels. Much additional experimental detail is required to have a clearer assessment of the potential relative importance of these models.

Between these extreme views (i.e., endocrine vs autocrine/paracrine), an important middle ground may have been neglected. Increasing evidence points to the likelihood that, even if the pathway is initiated by renin of kidney origin, a considerable portion of angiotensin II may be formed at a local level. In this construct, renin acts as the circulating "hormone" (i.e., a messenger from the kidney) which then initiates the synthesis of its effector molecule, angiotensin II, at or near its target cells. The evidence for widespread synthesis of ACE and of angiotensinogen, together with evidence for tissue-specific regulation of these proteins in normal and abnormal states, provides a compelling basis for the hypothesis that variations in these other components of the system may have significant impact on the regulation of angiotensin formation. Obviously, it is important to determine whether or not such local synthesis is driven by kidney renin, but it is equally important to consider that local variations in synthetic rate could lead to abnormal tissue levels of a short-lived peptide such as angiotensin II without having a feedback influence on the kidney. For example, according to the emerging studies of genetic variation in the renin-angiotensin system the deletion polymorphism of ACE, which is associated with increased risk of myocardial infarction (110), is associated with significantly higher plasma and tissue levels of ACE (111), including levels in the heart (112). Moreover, in one study, subjects harboring the deletion allele have an enhanced pressor response to exogenous angiotensin I (113), even though plasma levels of angiotensin II appear to be normal (114). These findings might suggest a mechanism leading to increased tissue angiotensin II levels without apparent effect on plasma levels of the peptide. In future studies, it will be important to clarify the validity and relative importance of each of these models in normal and pathological conditions, because further insight is likely to lead to more specific and effective therapies for cardiovascular disease.

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Left Ventricular Hypertrophy in Hypertension:

Etiology, Treatment, and Controversies

Robert A. Phillips, M.D., Ph.D., Joseph A. Diamond, M.D., and Ali Gharavi, M.D.

Cardiovascular Risk Associated with Left Ventricular Hypertrophy

Hypertensive heart disease is a common disorder associated with an increased risk of cardiovascular morbidity and mortality. Its manifestations include left ventricular (LV) systolic and diastolic dysfunction, coronary flow abnormalities, and arrhythmias. Echocardiographically determined hypertrophy of the left ventricle (LVH) is defined as the LV mass found in the upper 2.5 to 5% of the adult population. It occurs in approximately 15 to 20% of patients with hypertension (1). When considered as a discrete, categorical variable, LVH significantly increases the risk of coronary artery disease, congestive heart failure, cerebrovascular accidents, ventricular arrhythmia, and sudden death (2–4). LVH increases the relative risk of mortality by 2-fold in subjects with coronary artery disease and by 4-fold in those with normal epicardial coronary arteries (5, 6). In addition, when LV mass is considered as a continuous variable, a relatively linear relationship exists between cardiovascular risk and the absolute amount of LV mass. During 4 years of follow-up in the Framingham Heart Study, each 50 g/*M* increase in LV mass was associated with a 1.49 relative risk of cardiovascular disease for men and 1.57 for women. The effect on cardiovascular mortality was even more striking with a 1.73 relative risk for each 50 g/*M* for men and 2.12 for women (3).

This clinical review focuses on left ventricular hypertrophy, with a special emphasis on a critical review of the role of angiotensinogen and aldosterone.

LV Geometry in Hypertension: Etiology and Implications for Cardiovascular Risk

Patients with hypertension can be categorized by one of four patterns of ventricular geometry based on the presence or absence of LVH and the ratio of the ventricular wall thickness to the internal dimension [(interventricular septal thickness + the posterior wall thickness)/the internal dimension]. This ratio is defined as the relative wall thickness (RWT) (Table 1). RWT ≥ 0.45 is the cutoff often used to define concentric hypertrophy, a geometric pattern in which the LV internal dimension remains normal and LV mass increases because of increased wall thickness. In eccentric hypertrophy the LV internal dimension dilates and RWT is <0.45. This is the pattern typically associated with volume overload. Increased relative wall thickness with normal LV mass is termed concentric remodeling (7). In one study of subjects with hypertension observed for 10 years, the incidence of a cardiovascular event was 30% in those with concentric LVH, 25% in those with eccentric LVH, 15% in those with concentric remodeling, and 9% in those with normal LV mass (8).

Although concentric hypertrophy and remodeling are influenced largely by the degree of pressure overload, genetic factors may modulate the response. Several studies have shown that for the same blood pressure and LV mass. African Ameri-

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From the Hypertension Section, Cardiovascular Institute, Mount Sinai School of Medicine, New York, New York. Address correspondence to Robert A. Phillips, M.D., Ph.D., Director, Hypertension Section, Cardiovascular Institute, Box 1085, Mount Sinai School of Medicine, One East 100th Street, New York, NY 10029.

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TABLE 1
Patterns of Heart Geometry in Hypertension

Geometric pattern	LVH	RWT	Contributing factors[a]	10-year incidence of CV events (%)
Normal	Absent	<0.45		9
Concentric remodeling	Absent	>0.45	BP, TPR, race, aging, ACE	15
Concentric LVH	Present	>0.45	BP, TPR, race, ACE genotype	30
Eccentric LVH	Present	<0.45	Obesity with volume overload	25

[a] ACE, angiotensin-converting enzyme; BP, blood pressure; TPR, total peripheral vascular resistance.

can individuals with hypertension tend to have increased relative wall thickness compared with Whites (9–11). Hinderliter et al. (12) demonstrated that young adult Blacks without hypertension tend to have greater relative wall thickness than Whites, suggesting that differences in ventricular structure may be inherent. Gharavi et al. (13) recently demonstrated that polymorphism of the angiotensin-converting enzyme gene may influence LV remodeling. In that study, presence of the DD genotype, ambulatory systolic blood pressure, and race were each independently related to relative wall thickness (13).

Etiology of LVH

Because of its profound effect on cardiovascular morbidity, it is important to determine the factors that initiate LV growth. The effect of blood pressure, as well as virtually every factor known to influence blood pressure, has been investigated for its independent effect on LV mass (Table 1).

Strong evidence for a causal relationship between blood pressure and the absolute amount of LV mass was first reported more than 60 years ago (14). In the Framingham Heart Study (15), 10% of the variation in LV mass among subjects is accounted for by differences in systolic blood pressure averaged more than 30 years (15). Similarly, average blood pressure obtained during awake hours in subjects with hypertension accounts for 10 to 25% of LV mass variation (16–18), whereas the blunted nocturnal decline in blood pressure is associated with increased LV mass (19). In patients with hypertension approximately 40% ($r = 0.66$, $p < 0.001$) of the variation in LV mass is accounted for by total LV load or peak meridional wall stress (20).

The sequence of events that lead from increased wall stress to cellular hypertrophy is only beginning to be elucidated (21). It is likely that increased wall stress activates a stretch receptor, which, through a series of cellular and subcellular events, activates growth genes, such as c-*myc* and c-*jun*, to up-regulate myocardial cell protein synthesis (22). Myocardial protein degradation may also be suppressed by increased wall tension (23).

Clinical studies that suggest a role for "nonhemodynamic" influences on LV mass must be interpreted knowing that the characterization of the hemodynamic factors that affect LV mass, such as nocturnal blood pressure, may be incomplete. Nevertheless, a large body of data indicates that the degree of LV mass may be affected by many nonhemodynamic factors (Table 2).

Evidence supports a role for the genetic influence on LV mass. Epidemiological evidence for genetic influence on LV mass includes offspring studies that generally, but not uniformly, demonstrate that LV mass in children of parents with hypertension is elevated independently of blood pressure (61, 62). However, one twin study, in which monozygotic twins had only minimally less intertwin variation in wall thickness than dizygotic twins or sibling pairs, indicates that genetic influences on LV mass can be modified by environmental factors (56). Further evidence for a genetic influence on LV mass is that race seems to be a determinant of ventricular structure (discussed above) (63). Blacks have a greater total hemodynamic burden because the normal decrease in nocturnal blood pressure is usually blunted, compared with Whites, which, among other factors, might account for an increased LV mass and RWT (64). This altered blood pressure pattern begins in adolescence, and whether this is "genetic" or environmental remains to be determined.

The role of angiotensin II and aldosterone in hypertensive end-organ pathophysiology has been explored extensively. Some experimental and human studies (43, 53) have linked plasma renin activity to the degree of LV hypertrophy, but this is not accepted universally (54, 55). The synthesis of aldosterone, which is partially controlled by angiotensin II levels, appears to regulate cardiac fibroblast metabolism and growth (46). In primary hyperaldosteronism, the plasma aldosterone level has been related directly to LV mass (47). This may be mediated partially

TABLE 2
Association between LV Mass and Hemodynamic and Nonhemodynamic Factors

Factor	Strength of evidence supporting a causal role in LV mass[a]
Blood pressure/wall stress	Very strong (14–20)
LV volume	Very strong (20)
Obesity	Very strong (24–28)
Growth hormone and IGF-1	Strong (29–31)
Gender	Strong (32–34)
Race (concentric remodeling, not increased mass)	Strong (9–12, 35)
Age	Strong [women only?] (26, 36–39)
Alcohol	Needs confirmation (8)
Intrinsic myocardial contractility	Needs confirmation (20)
Blood viscosity	Needs confirmation (40)
Insulin resistance	Needs confirmation (24, 41, 42)
Parathyroid hormone	Needs confirmation (43)
Angiotensin II	Needs confirmation (44, 45)
Aldosterone (increased blood volume, collagen synthesis)	Needs confirmation (45–47)
Polymorphism of the ACE gene	Very controversial (13, 48, 49)
Sodium intake	Needs confirmation (50)
Na^+/H^+ exchanger and $Na^+/K^+/Cl^-$ cotransport system	Needs confirmation (51)
Intracellular Ca^{2+}	Needs confirmation (52)
Plasma renin activity	Controversial (43, 53–55)
Norepinephrine	Controversial (53, 56–59)
Na^+/Li^+ exchanger	Controversial (51, 60)

[a] Numbers in parentheses are Reference citations.

through increased plasma volume associated with hyperaldosteronism. *In vitro* studies suggest that local release of angiotensin II in response to the mechanical stretch is a necessary permissive factor for induction of the hypertrophic growth response (44). Subjects with hypertension who have relatively elevated plasma angiotensin II levels in relation to sodium balance have greater LV mass (45). The product of renin activity, angiotensin I, is the substrate for angiotensin-converting enzyme (ACE). Approximately 50% of the variation in ACE levels can be explained by genetic factors, one of which is a deletion polymorphism in the gene that encodes for ACE. However, the relation between ACE polymorphism and LV mass is controversial. The Framingham study did not find a relation between LV mass and ACE genotype (65), whereas a smaller study in an ethnically diverse population demonstrated a relationship (13).

Hormones and factors that regulate general growth also may be involved in myocardial hypertrophy. For example, marked increases in LV mass occur in patients with acromegaly as a result of elevated growth hormone and insulin-like growth factor 1 (IGF-1) (29). IGF-1 levels are higher in patients with hypertension who have LVH (30). *In utero,* insulin is a trophic factor that causes macrosomia (66). Insulin levels and the degree of insulin resistance may modulate LV mass independently in subjects with hypertension (24, 41, 42). One explanation for this finding is that insulin resistance leads to increased levels of intracellular calcium, possibly because of decreased Na^+K^+-ATPase activity (67). Elevated intracellular calcium may be an important stimulus for myocardial actin and myosin protein synthesis (52). This ionic hypothesis (68) may also explain the association between parathyroid hormone levels and LV mass in patients with hypertension (43).

LV Mass Regression: Clinical Trials

Regression of LV mass with antihypertensive treatment has been demonstrated in nearly 500 clinical studies, but less than 10% have been double-blind, placebo-controlled studies (69). Because few of these studies have been long-term it has not been determined whether regression of LVH increases survival. It is unlikely, however, that LV mass regression will prove detrimental because LV function is not adversely affected and diastolic function often improves (70–76). In the treatment of mild hypertension study (TOMHS), in which there was LV mass regression, total cardiovascular events were reduced (77). Furthermore, in the Framingham study, subjects who had regression of LVH on ECG during the course of observation had better prognoses (78).

The determinants of LV mass regression have been evaluated. Given the strong relation between blood pressure and LV mass, it is not surprising that in most studies there is a relatively strong relationship between the degree of blood pressure reduction and the amount of LV mass regression (69). A meta-analysis of 39 double-blind, randomized trials found that the greater the reduction in systolic BP, the greater the regression of LV mass ($r = 0.46$, $p < 0.001$).

Whether specific drug classes can affect LV mass independently of blood pressure is controversial. One study of 115 subjects suggested that treatment for 6 months with the converting enzyme inhibitor ramipril at a dose that did not reduce blood pressure (1.25 mg/day) nevertheless induced LV mass regression (79). A recent meta-analysis of 37 randomized trials compared diuretics, β-blockers, calcium channel blockers, and ACE inhibitor. For equal level of blood pressure reduction and duration of treatment. ACE inhibitors reduced LV mass more than β-blockers (13% vs 6% reduction in LV mass, $p < 0.01$). A trend toward superiority of ACE inhibitors over diuretics (13% vs 7%, $p = 0.08$) was observed, but no significant difference was detected between ACE inhibitors and calcium channel blockers (13% vs 9%, $p = 0.12$). The results of this meta-analysis, however, have been challenged in two recent randomized trials which suggest that diuretics are as effective, if not more effective, than other drug classes for reducing LV mass. In the TOMHS trial, blood pressure was reduced by a combination of weight loss plus either placebo, or one of five antihypertensive drug classes (β-blocker, α-blocker, calcium channel blocker, converting enzyme inhibitor, and diuretic) (77). At 1 and 4 years, all groups demonstrated LV mass regression, confirming that weight loss in conjunction with blood pressure reduction reduces LV mass. Only the chlorthalidone group had greater LV mass regression than the weight loss/placebo group. This was accounted for by reduced internal dimension as well as reduced wall thickness. Similar results were reported recently by the VA Cooperative Study Group who found that hydrochlorothiazide had a greater effect on LV mass regression than other antihypertensive agents for equal levels of blood pressure reduction (80).

Drug Choice for Treatment of LVH

Which drugs should a physician choose for treatment of patients with LVH? The clinician can be confident that, with the exception of pure vasodilators, blood pressure control will lead to LV mass regression. Because only diuretics and β-blockers have reduced mortality in patients with hypertension, one could easily take the position that these low-cost treatments are the first drugs that should be used to reverse LVH (81).

When would it be justified to use more expensive calcium channel blockers and ACE inhibitors for the patient with hypertension who has LVH? If one strongly believes in meta-analysis over randomized trials, then ACE inhibitors could be justified as a first-line agent. If the patient has angina or atrial arrhythmias and is intolerant of β-blockers, treatment with calcium channel blockers is an alternative that will also decrease LV mass. If the patient has systolic dysfunction, survival clearly will be improved with use of ACE inhibitors (82–85). What if the patient has diastolic dysfunction? Unfortunately, at this time, there is no evidence to suggest that any drug class is better than another. This simply reflects our ignorance about how to treat this syndrome, because only 71 of two million Americans with this syndrome have been enrolled in randomized clinical trials (86).

Finally, ACE inhibitors may possess reparative properties (cardioreparative, "myoreparative," and vascular) which distinguish them from other agents. Not enough studies are available, however on which to base clinical decisions. For example, it has been shown recently that enalapril improves coronary flow reserve in patients with hypertension (87). By contrast, calcium channel blockers may not improve coronary flow reserve (88). Another study comparing a β-blocker (atenolol) to an ACE inhibitor (cilazapril) demonstrated improvement in resistance arteries (reduced media/lumen ratio) in patients taking ACE inhibitors for 1 year but not in those taking β-blocker drugs (89). This finding was not confirmed in a 1-year study that compared a calcium channel blocker (isradipine) and a converting enzyme inhibitor (spirapril). Despite significant LV mass regression with both treatments, neither drug reduced the resistance of arterial medial thickness relative to lumen diameter (90). Finally, ACE inhibitors may possess the ability to reverse myocardial fibrosis, and thereby repair myocardial matrix (91). Whether this will have any clinical relevance remains to be determined.

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Angiotensin II and Cardiac Remodeling

Willa A. Hsueh, M.D., Yung S. Do, Ph.D., and Raju Jeyaseelan, Ph.D.

Substantial evidence exists that the renin-angiotensin system plays a major role in the cardiac remodeling process. This process involves cardiomyocyte hypertrophy and progressive interstitial fibrosis (1, 2). Mechanical stretch enhances angiotensin II (AII) production by cardiomyocytes which then hypertrophy in response to AII by an autocrine mechanism (3). In nonstretched myocytes, AII has only an inconsistent myocyte hypertrophy effect; however, AII also can promote myocyte hypertrophy indirectly. Endothelin (ET) and norepinephrine (NE) are potent hypertrophic factors for cardiac myocytes, even in the absence of stretch (4, 5). AII stimulates NE from cardiac nerve endings and ET from endocardial and endothelial cells (6, 7). Cardiac fibroblasts also play an integral role in cardiac remodeling. Although the cardiomyocytes hypertrophy, the fibroblasts proliferate and increase their production of extracellular matrix (8, 9). These events increase the volume of fibroblasts and matrix relative to that of myocytes in the hypertrophied heart compared with the normal heart, which ultimately results in ventricular dysfunction and heart failure (10). These interstitial changes impair myocardial contractility, oxygenation, and metabolism (11). Angiotensin II AT_1 receptor mRNA levels and protein are prominently detectable in rat cardiac fibroblasts (12–14). We and others demonstrated that they mediate AII effects to promote cardiac fibroblast proliferation, early growth response gene expression, and production of extracellular matrix proteins (14, 15).

Fibronectin is an extracellular matrix protein of particular interest because its mRNA and protein have increased in several models of left ventricular hypertrophy (LVH) (16, 17). It also participates in the repair response after experimental myocardial infarction (16). When AII was chronically infused in the rat, a 15-fold increase in fibronectin mRNA and lesser fold increases in collagens I and III occurred (18). This peak occurred on day 3 of the infusion, with a subsequent decrease in message levels of all matrix proteins. Losartan inhibited this effect, but the α-receptor blocker, prazosin, did not, despite equal lowering of blood pressure, which suggests that hypertension was not totally responsible for these results. Fibronectin plays a prominent role in wound healing and contributes to organization and laying down of collagen (19). Accumulation of fibronectin is associated with myocyte hypertrophy in the heart (16, 17) and with changes in vascular smooth muscle cells to a hypertensive phenotype in the blood vessel (20). In cultured rat cardiac fibroblasts from the adult or neonate, AII (10^{-5}–10^{-7} M) stimulates fibronectin mRNA by 4-fold, although it does not have a significant effect on collagen I and III mRNA levels under the conditions studied (15). ET and NE has no effect on growth or fibronectin production in the cultured cardiac fibroblasts, whereas epidermal growth factor (EGF) stimulates growth but has no effect on matrix production. Thus, in contrast to some other growth factors, AII has dual effects to promote both growth and fibronectin production in the cardiac fibroblast.

From the Department of Medicine, Division of Endocrinology, Diabetes and Hypertension, University of California at Los Angeles School of Medicine, Los Angeles, California. Address correspondence to Willa A. Hsueh, M.D., UCLA School of Medicine, Division of Endocrinology, Diabetes, and Hypertension, 900 Veteran Avenue, Suite 24-130, Box 957073, Los Angeles, CA 90095-7073.

Key Words: angiotensin II, cardiac remodeling, vasoconstriction, cardiac myocytes, fibronectin, angiotensin-converting enzyme inhibition, osteopontin, nitric oxide

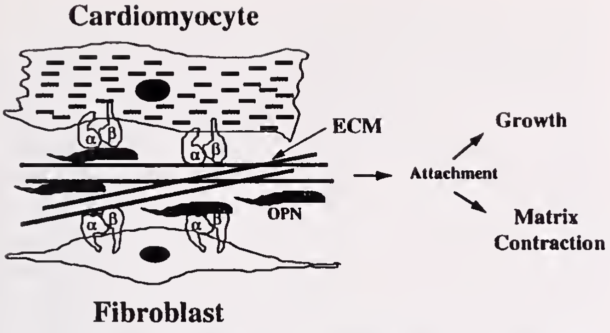

Fig. 1. Integrin receptors (α/β heterodimers) on the surface of cardiac fibroblasts and cardiomyocytes regulate cell interaction with the extracellular matrix. Osteopontin secreted by the fibroblasts and myocytes participates in that interaction, which ultimately regulates cell attachment to the matrix. Attachment is required for cell growth and matrix contraction, which represents the wound-healing response. ECM, extracellular matrix; OPN, osteopontin.

The importance of the direct cellular effects of AII is underscored by *in vivo* observations. Angiotensin-converting enzyme inhibitors (ACEI) both decrease and prevent the hypertrophic process resulting from hypertension by mechanisms apparently additive to the hemodynamic events associated with reduction of blood pressure (21, 22). Moreover, the progressive hypertrophy and dilation that occurs after myocardial infarction is attenuated by ACEI, which also decrease the incidence of severe congestive heart failures, recurrent myocardial infarction, and mortality after a coronary event in humans (21, 22). These results appear to be mediated by inhibition of AII because specific AT_1 receptor blockers decrease hypertrophy and interstitial fibrosis equally compared with ACEI in the rat heart after myocardial infarction (23). However, little more is known regarding the molecular and cellular mechanisms mediating AII effects on cardiac remodeling.

We recently discovered the presence of osteopontin mRNA and protein in the cardiac fibroblast and its prominent regulation by AII (24). Osteopontin is an acidic phosphoprotein adhesion molecule cloned from bone and recently identified in vasculature where it contributes to vascular smooth muscle cell (VSMC) migration, adhesion, and spreading, which are cellular functions in the vascular remodeling process that follow endothelial cell damage or stretch of VSMC (25, 26). Osteopontin contains an arginine-glycine-aspartic acid (RGD) motif which allows the molecule to bind to $\alpha_v\beta_3$ and other integrins on the surface of cells (27). An antibody to the β_3 subunit of $\alpha_v\beta_3$ integrin reduced the extent of restenosis in animals and in patients after angioplasty (28, 29), suggesting that alteration of integrin receptor activation has a significant impact on VSMC responses to injury. Osteopontin gene expression is regulated by AII and other growth factors in cultured rat VSMC (30). Osteopontin has not been identified previously in the heart, except in macrophages associated with inflammation and more recently in myocytes (31, 32).

We demonstrated that doses of AII as low as 10^{-11} M stimulated osteopontin mRNA in cultured rat cardiac fibroblasts; this effect is inhibited by the AT_1 receptor antagonist losartan. We further characterized other potential osteopontin receptors on the cardiac fibroblasts, which include α_v, β_1, β_3, and β_5 integrins. Preliminary results suggest AII can regulate gene expression of these integrins. To examine the function of osteopontin, we determined its effects on the ability of cardiac fibroblasts to proliferate and to contract three-dimensional collagen gels, which is a marker of fibroblast adhesion to collagen in wound healing. A monoclonal antibody against osteopontin partially blocked AII-induced collagen gel contraction by cardiac fibroblasts. Either a monoclonal antibody against β_3 integrin or the RGD peptide blocked both AII- and osteopontin-induced collagen gel contraction. Thus, the osteopontin RGD sequence binds to integrin on the fibroblast to promote its binding to collagen. AII (10^{-6} M for 24 hours) induced a 3-fold increase in DNA synthesis of cardiac fibroblasts. This growth effect was blocked completely by antibodies against osteopontin and β3 integrin or by RGD peptide, but not by control immunoglobulin G or RGE peptide. These observations suggest AII-induced growth of cardiac fibroblasts also requires osteopontin engagement of $\alpha_v\beta_3$ integrin receptor. Thus, osteopontin and $\alpha_v\beta_3$ integrin are required for attachment which is critical for growth and matrix adhesion (Fig. 1).

Consistent with our *in vitro* studies, we found that osteopontin mRNA and protein levels were up-regulated compared with their appropriate shams or controls in three rat models of left ventricular hypertrophy (LVH), aortic banding, 2 kidney-1-clip Goldblatt hypertension and the 5/6 nephrectomy uremic rat (24). In fact, ventricular atrial natriuretic peptide expression correlated with ventricular osteopontin expression (Fig. 2). We also found that cardiac fibroblasts taken from hearts of aortic banded rats demonstrated greater basal and AII-induced osteopontin expression and enhanced collagen gel contraction compared with fibroblasts from sham animals. More recently, immunohistochemical studies of hypertrophied ventricular tissue demonstrated the presence of osteopontin not only in cardiac fibroblasts, but also in cardiomyocytes (33). These results were

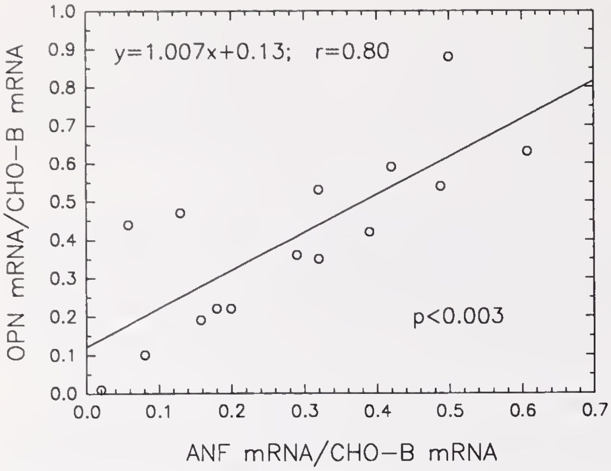

Fig. 2. Relationship of the expression of mRNA for osteopontin (OPN) and atrial natriuretic peptide (ANF) in ventricular cells from rats with LVH caused by aortic banding ($n = 7$) compared with sham-operated rats ($n = 7$). The results are normalized to the mRNA for CHO-B, a constitutively expressed gene, to correct for differences in loading or transfer of sample.

confirmed by *in situ* hybridization and in human hypertrophied ventricles. Osteopontin antibodies did not inhibit the hypertrophic response of cardiomyocytes, so the role of osteopontin in the myocyte remains unclear. One study suggests osteopontin may attenuate myocyte nitric oxide production (32). The present results suggest osteopontin is a paracrine factor which mediates myocyte signals that regulate cardiac fibroblast attachment, which then has an impact on fibroblast proliferation and matrix adhesion and contraction (Fig. 1). Recent evidence suggests cardiac fibroblasts produce a growth factor, possibly endothelin, which stimulates cardiomyocyte hypertrophy (34). Thus, the myocyte and fibroblasts maintain important communications which promote the hypertrophic remodeling process.

Whether AII has the same effects in human cardiac fibroblasts has been controversial because of the difficulty in identifying the presence of AT$_1$ receptors in human heart (35, 36). When we obtained cardiac fibroblasts from explanted human hearts, we identified the presence of AT$_1$ receptors in first and second passaged cells, but the presence of AT$_1$ receptor mRNA and protein rapidly decreased in third and further passages of the cells (Hsueh, unpublished observation). In second passaged cells, these receptors mediated protooncogene expression, DNA synthesis mitogen-activated protein kinase activity, and fibronectin and laminin production. All these effects were inhibited by an AT$_1$ receptor blocker, but not an AT$_2$ receptor blocker or an AT$_4$ receptor blocker. Thus, AII, through the AT$_1$ receptor, directly affects the promotion of growth and matrix production in human cardiac fibroblasts; these effects may account for the clinical observations that ACEI attenuate the cardiac remodeling process in patients with hypertension, postmyocardial infarction, and heart failure (20, 21). Our data suggest AT$_1$ receptor blockers should have similar effects as ACEI in patients with these forms of cardiac stress. Indeed, in a recent study comparing the AT$_1$ receptor blocker, losartan, with the ACEI, captopril, hemodynamic improvements and decreases in hospitalization were similar; however, mortality was lower with losartan than with captopril (37). Clearly, further studies are needed to define the role of AT$_1$ receptor blockers in human disease and the specific molecular and cellular effects of AII.

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Vascular Hypertrophy in Hypertension:

Role of the Renin-Angiotensin System

Clive Rosendorff, M.D., Ph.D.

Abstract

Angiotensin II is vasoconstrictor and antinatriuretic; it also stimulates cell growth and proliferation in vascular smooth muscle, resulting in hypertrophy or hyperplasia of conduit and resistance vessels. These actions are mediated through angiotensin II receptors (AT_1 subtype), which activate several G-protein-dependent intracellular transduction pathways, such as the phospholipase C, diacylglycerol and inositol trisphosphate pathways, the mitogen-activated protein (MAP) kinase pathway, and Janus kinase (JAK)-signal transducers and activators of the transcription (STAT)-mediated pathway. These can all increase the expression of certain proto-oncogenes, particularly c-*fos*. Angiotensin II also stimulates the activity of certain growth factors, such as platelet-derived growth factor-A-chain and basic fibroblast growth factor. The cellular responses to angiotensin II in vascular smooth muscle have been shown in different hypertensive vessels to be either hypertrophy alone, hypertrophy and DNA synthesis without cell division (polyploidy), or DNA synthesis with cell division (hyperplasia). In genetic hypertension, there is either cellular hyperplasia or remodeling, whereas in renovascular hypertension, there is hypertrophy of vascular smooth muscle cells. Angiotensin-converting enzyme (ACE) inhibitors prevent or reverse vascular hypertrophy in animal models of hypertension. In human hypertension, ACE inhibitors reduce the increased media/lumen ratio of large and small arteries and increase arterial compliance. These properties are also shared by AT_1 receptor antagonists. The implications of these findings for morbidity and mortality in hypertension still await rigorous testing in prospective clinical trials. **Key Words:** renin, angiotensin, vascular hypertrophy, hypertension, artery, angiotensin-converting enzyme, ACE inhibitor, angiotensin receptor, losartan.

ARTERIAL BLOOD PRESSURE CONTROL is mediated by a complex interaction of hormonal, neural, and metabolic factors. Neuroendocrine components maintain a balance between vasoconstrictor substances, such as angiotensin II, endothelin, the sympathoadrenal system, vasopressin, thromboxane and serotonin, and vasodilator substances such as atrial natriuretic peptide, nitric oxide, prostaglandins E_2 and I_2 (prostacyclin), the kallikrein-kinin system, and dopamine.

Some vasoconstrictor systems, such as the renin-angiotensin system, are also antinatriuretic and antidiuretic, which augments their blood pressure-raising action. In addition, there is another characteristic of these vasoconstrictor-antinatriuretic hormones that recently has been the subject of considerable interest and research, namely that they may operate as growth factors in the myocardium and in vascular smooth muscle, affecting growth-related genes to promote cell hyperplasia, hypertrophy, or both (1–3). At least some of the vasodilator hormones have the opposite effect, being natriuretic and also inhibiting cellular proliferative and growth processes.

This review examines some of the more cogent evidence that angiotensin II stimulates vas-

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From the Department of Medicine, Mount Sinai School of Medicine, New York, NY, and Medical Service, Veterans Affairs Medical Center, Bronx, NY. Address correspondence to Dr. Clive Rosendorff, Medical Service (111), Veterans Affairs Medical Center, 130 West Kingsbridge Road, Bronx, NY 10468.

cular hypertrophy and hyperplasia, that this may be important in the pathogenesis of hypertension, and that angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor antagonists are vasoprotective, limiting or even reversing the vascular morphology more than can be accounted for by blood pressure reduction alone.

The Renin-Angiotensin System

The major components of the renin-angiotensin system are angiotensinogen, renin, angiotensin I, ACE, and angiotensin II.

Angiotensinogen, a large globular protein, is synthesized in the pericentral zone of liver lobules (4). The glycoproteolytic enzyme, renin, cleaves a leucine-valine bond in the N-terminal region of human angiotensinogen to produce the decapeptide angiotensin I (4, 5). The major source of renin is the juxtaglomerular cells of the afferent arteriole of the kidney. Translation of renin mRNA in these cells produces pre-prorenin, which in turn is converted to prorenin. Some prorenin-to-renin conversion takes place in the juxtaglomerular cells, and both are secreted. Prorenin is the more abundant circulating form of renin, however; the major site of conversion of prorenin to renin is unknown. Prorenin mRNA is expressed at very low levels (5) or is absent (6) in blood vessels, but there is avid uptake of prorenin by vascular tissue, which suggests that blood vessels may be the primary site of the formation of renin from circulating prorenin. Some controversy exists whether renin is synthesized to any significant extent in cardiovascular tissue or is derived entirely from plasma uptake.

Angiotensin-converting enzyme converts angiotensin I to the octopeptide angiotensin II, and also inactivates bradykinin. Bradykinin stimulates the release of vasodilating protaglandins (7) and nitric oxide (8), and may be responsible for ACE inhibitor-induced cough (9). Angiotensin-converting enzyme has been found in nearly all mammalian tissues. Conflicting evidence exists whether vascular ACE is increased in experimental hypertension; some reports have shown an increase in vascular ACE in two-kidney, one-clip hypertensive rats (10, 11) but this has not been confirmed in the spontaneously hypertensive rat (SHR) (12, 13).

Some enzymatic pathways independent of ACE [tissue-type plasminogen activator (t-PA), cathepsin, tonin, and elastase] allow for the formation of angiotensin II directly from angiotensinogen (14). Enzymes other than ACE [t-PA, tonin, cathepsin G, chymase, and a chymostatin-sensitive angiotensin II generating enzyme (CAGE)] catalyze the formation of angiotensin II from angiotensin I (14, 15). The importance of these pathways is obscure, and, in particular, it is not known whether these non-ACE pathways are present *in vivo,* or whether they are activated only when the conventional ACE pathway is blocked. There is also little or no experimental evidence that ACE-independent pathways contribute substantially to angiotensin II biosynthesis or to vascular hypertrophy.

Angiotensin II Receptors

Two major angiotensin II receptor types exist, AT_1 and AT_2 (16, 17). The AT_1 receptors are found in vascular and many other tissues, and are almost certainly the receptors that transduce angiotensin II-mediated cardiovascular actions, as discussed in the next section.

Less is known about AT_2 receptors. The AT_2 binding sites are much more abundant in fetal and neonatal than in adult tissue, suggesting a role in development (18, 19). Localization is mainly in the brain (20). It is probable, therefore, that AT_2 receptors have little to do with the acute cardiovascular actions of angiotensin II. Also, as described later, most of the growth-promoting effects of angiotensin II on arteries seem to be mediated by AT_1 receptors.

The involvement of the AT_2 isoform of the angiotensin II receptor in myointimal hyperplasia after vascular injury has been reported (21). A very interesting finding in another study was that AT_2 receptors were up-regulated in quiescent vascular smooth muscle cell cultures that had been suppressed by serum depletion. In contrast, stimulation of the cells by growth factors, such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), or serum, rapidly down-regulated the AT_2 receptor (2, 22). These results suggest that AT_2 receptor expression parallels the suppression of vascular smooth muscle cell growth, the reverse of AT_1 receptors. How these effects balance each other and how this balance is upset in angiotensin II-induced vascular hypertrophy is a new and potentially important area of research.

Angiotensin II Signal Transduction Pathways for Mitogenesis and Growth

The AT_1 receptors are present in vascular and many other tissues and seem to mediate the vasoconstrictor and growth stimulation effects of

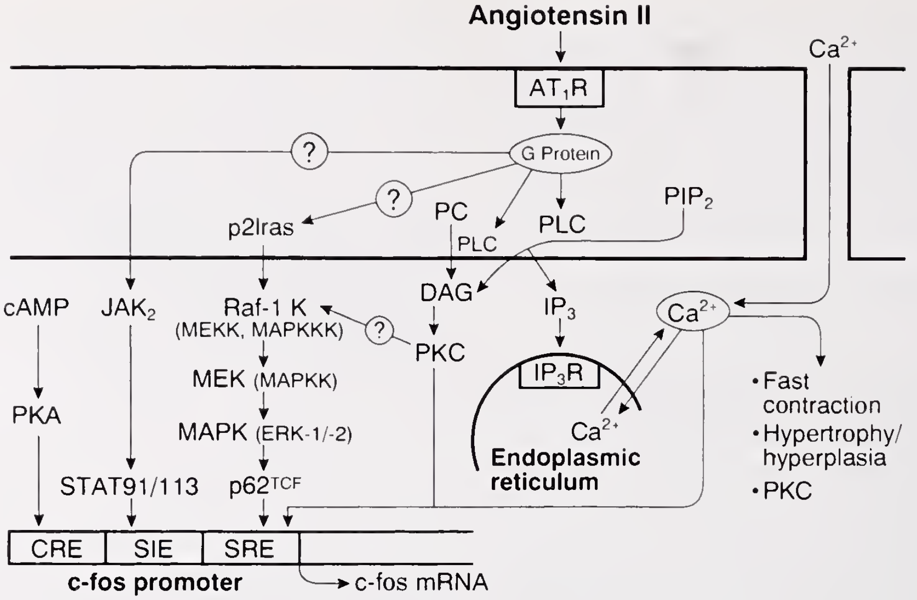

Fig. Signal transduction pathways for the angiotensin II receptor (subtype AT_1). The receptor is coupled to a guanine nucleotide-binding regulatory protein (G protein), which activates phospholipase C (PLC). PLC catalyzes the hydrolysis of phosphatidylinositol bisphosphate (PIP_2) to inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG). Inositol 1,4,5-trisphosphate, acting through the IP_3 receptor (IP_3R) on the endoplasmic reticulum, stimulates the mobilization of Ca^{2+} from intracellular stores, a process also accelerated by the influx of Ca^{2+} through voltage-dependent Ca^{2+} channels during activation (Ca^{2+}-dependent Ca^{2+} release). Free cytosolic Ca^{2+} has many actions relating to contractility and cell hypertrophy or hyperplasia including the activation of protein kinase C (PKC). An alternative pathway for the formation of DAG is through the hydrolysis of phosphatidylcholine (PC) by PLC. DAG activates PKC, which in turn may induce hypertrophy or hyperplasia through several mechanisms, one of which is the activation of a serum response element (SRE) on the c-*fos* promoter. The SRE also interacts with products of the MAP kinase phosphorylation cascade. Both PKC and a small-molecular-weight guanine-nucleotide-binding protein, p21ras, regulate the serine/threonine kinase Raf kinase (Raf-1 K) which acts as a MEK kinase (or MAP kinase kinase kinase). MEK (MAP/ERK Kinase) is a MAP kinase kinase, and the mitogen-activated protein (MAP) kinase has two active isoforms ERK-1 and ERK-2 (extracellular-signal-regulated kinases). Activated MAP kinase substrates include the transcription factor $p62^{TCF}$, which forms a complex on the c-*fos* promoter (SRE). Angiotensin II also stimulates the phosphorylation and activity of STAT 91 and STAT 113 through the action of Janus kinase 2 (JAK 2); this interacts with a sis-inducing element (SIE) on the c-*fos* promoter. Another c-*fos* promoter element is a cAMP response element (CRE) which is sensitive to protein kinase A (PKA). The significance of this pathway in angiotensin II cell signaling is not known.

angiotensin II in vascular smooth muscle. The AT_1 receptor is coupled to a guanine nucleotide-binding regulatory protein (G protein) which activates phospholipase C (PLC). PLC catalyzes the hydrolysis of phosphatidyl inositol bisphosphate (PIP_2) to inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG) (Figure). IP_3, acting through the IP_3 receptor (IP_3R) on the endoplasmic reticulum, stimulates the mobilization of Ca^{2+} from intracellular stores, a process accelerated also by the influx of Ca^{2+} through voltage-dependent Ca^{2+} channels during activation (23, 24). The increase in cytosolic Ca^{2+} concentration is an essential component in both the activation of the contractile proteins of vascular smooth muscle, and in mediating the growth-promoting actions of angiotensin II and other growth factors, at least partially through protein kinase C (PKC) activation (25).

An alternative pathway for the formation of DAG exists through the hydrolysis of phosphatidylcholine (PC) by PLC or by phospholipase D (PLD). Both DAG and Ca^{2+} activate a PKC which has many actions. PKC affects transmembrane Na^+/K^+ exchange to alkalinize the cytoplasm which is important in mitogenesis. PKC activates a serum response element (SRE) found on the promoter region of c-*fos,* an early-response proto-oncogene activated by angiotensin II, and thought to be a major factor in initiating the nuclear events that result in cell proliferation and growth.

An increasing number of publications suggest that there are alternative signal transduction pathways for angiotensin II (26–29). One of these is the mitogen-activated protein (MAP) kinase cascade. Although many components of this pathway have been identified, it is not known how angiotensin II (which binds to a G-protein-coupled receptor that lacks intrinsic tyrosine kinase activity) feeds into the MAP kinase phosphorylation cascade. One possibility is through PKC regulation of Raf-1 kinase (30). Convincing evidence, nevertheless, shows that the MAP kinase pathway mediates some of the vascular growth promoting actions of angiotensin II.

Binding of angiotensin II to the G-protein-coupled AT_1 receptor activates small-molecular-weight guanine-nucleotide binding protein, p21ras (26, 27), which activates the serine/threonine kinase, Raf-1 kinase, or the MEK (MAP or extracellular-signal-regulated kinase (ERK) kinase) kinase. MEK kinase phosphorylates MEK (also called MAP kinase kinase), a tyrosine/threonine kinase, which in turn activates the serine/threonine kinase, MAP kinase, of which the most important forms in this pathway are p44 (ERK 1) and p42 (ERK 2). The ERK isoforms of MAP kinase are responsible for the phosphorylation of a variety of proteins *in vitro* including the kinase that phosphorylates ribosomal S6 proteins $pp90^{rsk}$ (31), and the transcription factors c-*jun* and $p62^{TCF}$ (also called elk-1), which forms a complex at the SRE on the c-*fos* promoter. Other MAP kinases include JNK (c-*jun* kinase, also called SAPK, or stress activated protein kinase), responsible for c-*jun* activation, and p38, which may also activate nuclear proto-oncogenes like c-*fos*, c-*myc*, and c-*jun*.

As if this level of complexity were not enough, there is a third possible pathway for angiotensin signal transduction. Angiotensin II stimulates the phosphorylation and activation of members of the signal transducers and activators of transcription (STAT) family of transcription factors (26, 31) through the action of Janus kinase 2 (JAK 2). The STAT factors, particularly STAT 91 and STAT 113, activate a sis-inducing element (SIE) on the promotor region of c-*fos*.

We still do not know the extent to which these signal transduction pathways are shared by receptors which mediate vasoconstriction and, possibly, vascular hypertrophy, such as AT_1, α-adrenergic, and endothelin receptors. We also do not know much about the physiologic specificity of these pathways, such as which are essential for cell hypertrophy vs hyperplasia, which activate c-*fos*, c-*jun*, or c-*myc* selectively, and which of the myriad of intracellular events activated by angiotensin II depend on which pathway. It is obvious, however, that this is an area of research in which there is enormous potential for the development of new gene and pharmacologic therapies for many clinical problems, with great precision in modifying very specific cell processes.

Atherogenic Effects of Angiotensin II

Depending on the animal model studied (32), angiotensin II can produce vascular smooth muscle hypertrophy alone, hypertrophy and DNA synthesis without cell division (polyploidy), or DNA synthesis with cell division (hyperplasia). For example, cells from normotensive rats become hypertrophied when incubated with angiotensin II (33), whereas angiotensin II induces a proliferative response in cells from SHR (34, 35). The data from whole animal studies, i.e., animal models of experimental hypertension, are consistent with the cell culture results. Vascular smooth muscle hypertrophy has been reported in renal hypertension (angiotensin II-dependent) (36) and angiotensin II-induced hypertension (37). In genetic models of hypertension such as in SHR, however, there is cellular hyperplasia (38), or in low-renin transgenic rats (39) and essential hypertension in humans (40), there is a redistribution of existing cells referred to as remodeling.

These different effects of angiotensin II on different cell and animal models of hypertension are difficult to explain. Several lines of evidence suggest, however, that angiotensin II stimulates both proliferative and antiproliferative cell processes. The proliferative actions include the stimulation of the growth factors platelet-derived growth factor-A-chain (PDGF-A) and basic fibroblast growth factor (bFGF), possibly via AT_1 receptors. The antiproliferative processes include transforming growth factor-β1 (TGF-β1) (41, 42). Another antiproliferative mechanism is the ability of the AT_2 receptor to mediate programmed cell death (apoptosis) by dephosphorylation of MAP kinase (43), or to inhibit guanylate cyclase (44).

Angiotensin II also has a profound effect on the composition of the extracellular matrix of vascular smooth muscle cells, including the synthesis and secretion of thrombospondin, fibronectin, and tenascin (45). Other processes of atherogenesis are stimulated by angiotensin II, such as migration of vascular smooth muscle cells (46), the activation, release of tumor necrosis factor-α (TNF-α), and the adhesion to endothelial cells by human peripheral blood monocytes (47), and thrombosis. Angiotensin II increases plasminogen activator inhibitor type 1 in rat aortic smooth muscle cells (48) and in humans *in vivo* (49). All of these actions increase the probability that angiotensin II is atherogenic and prothrombotic, and that ACE inhibitors or angiotensin II antagonists may exert some protective effect through these mechanisms.

Effect of ACE Inhibitors on The Structure of Arteries

In hypertension, there are consistent and major changes in the structural and functional properties of arteries and arterioles (50–52), causing an increase in arterial resistance and stiffness. These changes include:

- a reduction in the external and internal diameter of the vessel wall, without any increase in its cross-sectional area, known as remodeling;
- altered wall thickness, with medial hypertrophy, myointimal proliferation, and an increase in collagen content;
- increased passive stiffness of the vessel wall, probably caused by the increase in collagen and smooth muscle mass; and
- increased active vascular muscle tone, caused by a variety of local and extrinsic metabolic and neurohormonal factors. For example, two-kidney one-clip Goldblatt hypertensive rats that have increased levels of plasma renin activity and angiotensin II had increased aortic impedance, decreased arterial compliance, and thickening of these vessels, caused by smooth muscle hypertrophy, all reversed by the ACE inhibitor perindopril (53, 54). Similar results have been described for young SHRs treated with captopril (55), and for perindopril (56) or cilazapril-treated (57) adult SHRs, in which plasma levels of renin activity, angiotensin II, and renal renin content are not different from normotensive Wistar Kyoto control rats (58, 59).

Is the prevention of vascular hypertrophy by ACE inhibitors in these animal models of hypertension unique to this class of antihypertensive agents, or is it a nonspecific consequence of blood pressure reduction? Pure vasodilators, such as hydralazine, which increase the plasma level of angiotensin II, do not prevent vessel wall thickening in SHRs, despite the normalization of blood pressure (60), and ACE inhibitors have been shown to be more effective than other antihypertensive agents (β-blockers, vasodilators) in decreasing vascular hypertrophy, despite similar decreases in blood pressure (61, 62). Not all investigators have been able to confirm this; Hadju et al. (63) reported that in stroke-prone SHRs, cilazapril increased both the internal and external diameter of cerebral arterioles, whereas hydralazine reduced hypertrophy, but with little increase in the external diameter.

Do the various ACE inhibitors differ in their ability to prevent or reverse vascular remodeling or hypertrophy? Frohlich and Horinaka (64) found in SHRs and Wistar Kyoto rats that six ACE inhibitors (captopril, CGS-16617, cilazapril, enalapril, utibapril and quinapril) had similar effects on systemic hemodynamic variables. However, there was considerable variability among the drugs in their ability to affect aortic hypertrophy and distensibility as well as left ventricular mass and performance. These differences could reflect their pharmacodynamic (e.g., enzyme inhibition) and pharmacokinetic (e.g., cell penetration) properties.

The role, if any, of bradykinin in the regression of vascular hypertrophy by ACE inhibitors has not been determined. Linz et al. (65) reported that the antiproliferative actions of ramipril in cholesterol-fed rats were abolished by HOE 140, a selective B_2-bradykinin receptor antagonist. A possible mechanism of this effect is bradykinin-induced release of nitric oxide from endothelial cells which has an antiproliferative effect. However, HOE 140 did not block the antihypertrophic effect of ramipril in renal hypertensive rats with aortic banding.

ACE Inhibition and Vascular Structure and Function in Human Hypertension

Studies in human subjects with hypertension fall into two broad categories. The first involves biopsy of subcutaneous fat in the gluteal region to measure structural and functional properties of small arteries, and the second, somewhat less invasive, measures large (e.g., brachial) artery pressure and diameter (by ultrasound) to calculate arterial compliance.

At least two important studies, using gluteal biopsy, show the effects of ACE inhibitors on vascular morphology. Schiffrin et al. (66) compared the action of the ACE inhibitor cilazapril with the β-blocker atenolol administered for 2 years to 2 groups of patients with hypertension. Cilazapril normalized the media/lumen ratios of the small subcutaneous arteries, but there was no change with atenolol. Thybo et al. (67), also using gluteal biopsy, showed that treatment of patients with hypertension for 12 months with perindopril resulted in an increase in small artery diameter and a reduction in the ratio of media thickness/lumen diameter, but no change in the media cross-sectional area, suggesting that the change was caused by remodeling.

One of the most consistent findings in hypertension, whatever the stage of the disease or the site of measurement, is arterial "stiffness," or a decrease in arterial compliance. This is true of borderline hypertension (68), sustained hypertension in middle-aged patients (69), and in systolic hypertension in older people (70). Treatment with ACE inhibitors both increases arterial diameter and improves compliance (71, 72), not simply because of flow-dependent dilation, but because of drug-related relaxation of arterial smooth muscle (73).

Because of the difference in loading of elastin and collagen in the walls of large arteries, the pressure-volume relation in these vessels is not linear (74). Thus, the relationship, measured by ultrasound, between the diameter of the artery and blood pressure, reveals that the compliance pressure curve is nonlinear. The higher the arterial pressure, the lower the compliance. Therefore, all drugs that lower blood pressure increase compliance as a simple mechanical consequence of pressure reduction. Some, but not all, antihypertensive drugs, however, increase compliance by amounts greater than can be predicted on the basis of their pressure-lowering effect; the compliance-pressure curve is shifted upward. Essentially, this means that all antihypertensive drugs reduce arterial blood pressure by reducing arteriolar resistance, but only some dilate large arteries as well.

Generally, ACE inhibitors (75, 76) and calcium channel blockers (77, 78), improve large-artery compliance in hypertension, whereas the thiazide diuretic drugs (71, 76), urapidil (79), ketanserin (80), and clonidine (81) do not. Another group of investigators (82, 83) measured radial artery compliance in normotensive subjects before and after 8 days of treatment with placebo atenolol, nitrendipine, or lisinopril. Only lisinopril shifted the compliance-pressure curve upward.

Another approach to the assessment of vascular resistance and distensibility, in this case of small precapillary resistance vessels, uses forearm and hand venous occlusion plethysmography to measure maximal blood flow after ischemia, from which forearm minimal resistance can be calculated. Using this technique, several workers have described decreases in minimal resistance after 6 months of therapy with pindolol, captopril, or nitrendipine (84–86). In a double-blind, randomized, parallel study, Dahlöf and Hansson (87) showed that after 6 months of therapy, captopril reduced forearm minimal resistance, whereas hydrochlorothiazide did not.

Although many studies have been done on the effects of therapy on arterial compliance in patients with hypertension there have been very few on changes in vascular morphology, as assessed by noninvasive methods such as B-mode ultrasound. One such study, reported recently (88, 89), showed an equal decrease in radial wall thickness in patients with hypertension treated for 9 months with either perindopril or hydrochlorothiazide plus amiloride. The Perindopril Regression of Vascular Thickening European Community Trial (PROTECT) will compare the effect of perindopril with hydrochlorothiazide on carotid artery intima/media thickness in 800 young to middle aged patients with hypertension (90).

Angiotensin II Receptor Antagonists

A major advance in antihypertensive drug therapy has been the development of nonpeptide angiotensin II receptor antagonists selective for the AT_1 receptor subtype, which mediates the vasoconstrictor actions of angiotensin II. A critical question is whether the hypertrophic action of angiotensin II can also be inhibited by selective AT_1 receptor antagonists. Losartan, the first AT_1 receptor antagonist developed, blocked angiotensin II-induced DNA and protein synthesis and intracellular Ca^{2+} mobilization in cultured rat aortic smooth muscle cells, whereas PD 123177, an AT_2 receptor antagonist, had no effect (91). Angiotensin II-induced increases in cell size, protein content, and c-*fos* induction in cultured arterial smooth muscle cells were inhibited by losartan but not by CGP 42112A, an AT_2 receptor blocker (92). Losartan blocks angiotensin II stimulation of DNA synthesis (93), c-*fos* expression (94), and the phosphoinositide-signaling system (95).

In intact animals, results with losartan have been consistent with those from cell culture. Losartan reduced medial thickness in the aorta and mesenteric arteries in growing SHRs (96), in the aorta and tail arteries of 24-week SHRs after only 2 weeks of treatment (97), in mesenteric arteries in 3-week-old SHRs (98), and in mesenteric, renal, coronary, and femoral arteries in 4- to 20-week-old SHRs (99). Another study on stroke-prone SHRs (100) found only modest reductions in wall/lumen ratio in the middle cerebral arteries after 15 weeks of therapy with a high dose of losartan, but there was a profound improvement in blood pressure, arterial contractility, endothelial function, and stroke mortality. Morishita et al. (101) transfected the human ACE gene into intact rat carotid arteries. An increase in local ACE activity was associated with a parallel increase in DNA synthesis, protein content, and wall/lumen ratio, all abolished by the administration of losartan. These effects of locally generated angiotensin II, and their reversal by losartan, occurred without any changes of circulating angiotensin II or arterial blood pressure, supporting the concept that increased autocrine/paracrine angiotensin II can directly cause vascular hypertrophy, independent of systemic factors and hemodynamic effects.

Conclusion

It is now reasonably well established that ACE inhibitors and AT_1 receptor blockers reduce wall thickness or increase compliance of large arteries in various animal models of hypertension and in humans. Many questions remain about the significance of these findings. Is the improved compliance a measure of the regression of vascular smooth muscle hypertrophy, or of a decrease in the elastin-collagen matrix, or both? Are the wall thickness changes caused by regression of early atherosclerotic lesions, remodeling of the hypertrophied media, a decrease in the number of individual smooth muscle cells, or a combination of these? To what extent do these changes reflect those that may or may not be occurring in small-resistance arteries and arterioles, beyond the reach of conventional ultrasound? What do these data, obtained mainly from brachial and common carotid arteries, tell us about the coronary, intracerebral, and intrarenal arteries? Are these improvements in functional and morphologic properties of large arteries markers for improved clinical outcomes?

It is an intuitive and attractive idea that it is advantageous to increase vascular compliance and to reverse vascular (and left ventricular) hypertrophy, and that agents such as ACE inhibitors and AT_1 receptor blockers, which do this better than other antihypertensive agents, might improve morbidity and mortality to a greater extent than can be expected on the basis of the blood pressure reduction alone. At least one study is under way to answer this question (102).

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The Renin-Angiotensin System as a Mediator of Renal Injury in Hypertension

Ali Gharavi, M.D., and Paul Klotman, M.D.

Hypertension is both a cause and a consequence of kidney disease. Essential hypertension is a strong risk factor for the development of end-stage renal disease (1). However, the molecular basis for hypertension-induced renal failure is complex. Potential mechanisms for pathogenesis include hemodynamic stress, growth factor dysregulation, and neurohormonal factors (1). Because the kidneys are the long-term regulators of systemic blood pressure, renal diseases are a common secondary cause of hypertension (2). The renin-angiotensin system (RAS) is one of the best studied neurohormonal systems related to hypertension and kidney disease. Through the regulation of blood pressure and intravascular volume, the circulating RAS indirectly can cause renal injury. In addition, recent evidence suggests that the RAS can contribute directly to renal damage through nonhemodynamic mechanisms. For example, angiotensin II is a growth factor that stimulates cell growth and extracellular matrix production in many tissues (3). Furthermore, all components of the RAS are expressed in the kidney, heart, and vasculature, which allows local generation of angiotensin II (4). The intrarenal autocrine-paracrine RAS is regulated differentially and operates independently to alter renal microcirculation and cell growth (5–7). Animal and human studies have also shown that pharmacologic interruption of the RAS offers renoprotection beyond its hemodynamic benefits (8, 9). These data strongly suggest that angiotensin II at the tissue level may be a critical factor in the pathogenesis of many renal diseases. Investigators have also suggested that the circulating RAS is responsible for acute hemodynamic adaptation, whereas the local RAS is involved in long-term regulatory mechanisms (10). This review will discuss our current understanding of the RAS as a mediator of renal injury and its potential role in the development of hypertensive nephrosclerosis.

Characteristics of the Intrarenal RAS

Expression and Regulation

Studies have shown that all the components of the RAS (renin, angiotensinogen, and angiotensin-converting enzyme [ACE]) are expressed locally and regulated differentially in the kidney (3). For example, intrarenal angiotensin II levels are up to 1,000 times higher than the plasma level (11). In rats, pressor doses of renin decrease plasma angiotensinogen levels and suppress angiotensinogen transcription in the liver and the hypothalamus (12). In the kidney, however, angiotensinogen mRNA levels remain unchanged (11). Pressor doses of angiotensin II in rats reduce systemic and renal renin levels but increase angiotensinogen transcription in the liver and the kidney (5). The up-regulation of hepatic and renal angiotensinogen is not abolished by an angiotensin-converting enzyme inhibitor (CEI), suggesting a direct effect of angiotensin II on angiotensinogen transcription (5). In rats with compensated experimental heart failure, renal angiotensinogen is up-regulated selectively despite normal cir-

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From the Department of Medicine, Division of Nephrology, Mount Sinai School of Medicine, New York, New York. Address correspondence to Paul Klotman, M.D., Professor of Medicine, Division of Nephrology, Mount Sinai School of Medicine, Box 1243, One East 100th Street, New York, NY 10029.

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culating RAS parameters, which suggests a distinctive role for the renal RAS in the pathophysiology of heart failure (6). There is also evidence that ACE is regulated differentially in the kidney. ACE is present in the brush border of renal proximal tubules (13), and it is up-regulated selectively in the nonclipped kidney during the maintenance phase of the two-kidney, one-clip Goldblatt hypertensive rat model (14).

The mechanisms regulating the intrarenal RAS must still be fully elucidated. As noted above, circulating angiotensin II itself augments intrarenal angiotensin II levels. Direct infusion of angiotensin II in uninephrectomized rats results in marked elevation of intrarenal angiotensin II levels and causes glomerulosclerosis (15). Administration of an angiotensin II receptor antagonist leads to a further increase in systemic angiotensin II levels but prevents the increase in intrarenal levels and reduces glomerular injury (14). The maintenance phase of the two-kidney, one-clip Goldblatt model of hypertension also is characterized by an increased angiotensin II level in the nonclipped untouched kidney (16). This occurs despite normal circulating levels of angiotensin II and suppression of renal renin content in the untouched kidney (17), which suggests that angiotensin II is generated via up-regulation of ACE or other renin-like substances (13). In fact, Yosipov et al. (19) recently have shown that newborn and adult rat kidneys possess angiotensin-generating enzymes distinct from the traditional component of the RAS. The enzymes may generate angiotensin II directly from angiotensinogen, raising the possibility that they could participate in the regulation of the tissue RAS in pathologic states.

Other regulators of intrarenal RAS include endothelin-1 (ET-1) and the sympathetic nervous system. Renal sympathetic nerve activity stimulates renal renin and angiotensinogen expression; this response is diminished in spontaneously hypertensive rats, an animal model of hypertension (19). Angiotensin II also stimulates (ET-1) release from many renal cell types in culture, but little information is available on *in vivo* interaction (reviewed in ref. 3). Finally, increased blood pressure and wall tension may be other potential activators of the intrarenal RAS. Hemodynamic forces can elicit a cellular response via activation of stretch receptors. Mechanical stretch induces autocrine production of angiotensin II in cardiac tissue and in the vascular wall (20, 21). Pressure also stimulates ET-1 release from human endothelial cells (22). Because mesangial cells and vascular smooth muscle cells share many similarities, it is conceivable that higher systemic blood pressure can directly stimulate secretion of angiotensin II or other vasoactive substances by glomerular mesangial cells.

Most of the effects of angiotensin II discussed in this paper are mediated by the angiotensin II type-1 (AT_1) receptor. This receptor is a classic G protein-coupled receptor with seven transmembrane domains (23). The details of the AT_1 receptor signal transduction pathway are still poorly understood but involve the activation of phospholipase C by tyrosine phosphorylation (24). This leads to downstream stimulation of protein kinase C and other protein kinase pathways implicated in the regulation of intracellular calcium stores (23). Angiotensin II can thus activate the cellular contractile apparatus by mobilization of intracellular calcium (23). Angiotensin II activation of protein kinases is also associated by increased expression of many proto-oncogenes, such as c-*fos,* c-*myc,* and *Erg*-1, reflecting the long-term actions of angiotensin II on cell growth (25–28). An angiotensin II type-2 (AT_2) receptor has also been identified (22). It is highly expressed in the late fetal stages and in early postnatal life, which suggests a role in development (28). The physiologic function of the AT_2 receptor in the adult organism is still unknown but emerging data in vascular smooth muscle cells suggest that it may mediate an antiproliferative response (29).

Angiotensin II and Intrarenal Hemodynamics

Angiotensin II induces smooth muscle cell contraction, leading to vasoconstriction and increased peripheral vascular resistance. It also acts in the central nervous system to stimulate thirst and promotes aldosterone release from the adrenal gland. In the kidney, angiotensin II enhances sodium reabsorption directly via activation of the Na/H^+ antiporter in the proximal tubule and indirectly via vasoconstriction of the vasa recta in the loop of Henley (30). The resulting constriction of the medullary vessels diminishes renal excretory capacity. Intrarenal infusion of angiotensin II in the dog reduces papillary blood flow and shifts the pressure-natriuresis relationship leading to decreased sodium excretion and the development of a salt-sensitive form of hypertension (31). In the glomerulus, angiotensin II constricts afferent and efferent arterioles but may act preferentially on the efferent arteriole, thereby regulating glomerular hemodynamics and filtration fraction (32, 33). Angiotensin II also induces mesangial cell contraction, leading to alterations in the glomerular ultrafiltration coefficient (34). Subpressor doses of angiotensin II increase glomerular permeability and decrease the clearance of macromolecules from the mesangium (35). Thus, high angiotensin II levels can alter glomerular hydraulics and size selectivity leading to increased proteinuria. Proteinuria itself may promote interstitial inflammation and renal injury. Therefore, renoprotective effects of converting enzyme inhibitors may be attributed partly to interruption of the hemodynamic effects of angiotensin II in the glomerulus.

Angiotensin II as a Growth Factor

Data show that angiotensin II is an important growth factor during renal development in the late fetal stages and early postnatal life. Angiotensin II levels and AT_1 receptor expression are related temporally to kidney growth and maturation (18, 36). Blockade of RAS action with AT_1 receptor antagonists in early postnatal life leads to tubulointerstitial inflammation and papillary and cortical atrophy (37). Other investigators have observed delayed maturation of the glomeruli after treatment of rats with CEIs (38). Similar renal developmental defects were observed in knock-out mice with disruption of the angiotensinogen gene (39). In cell culture experiments, angiotensin II induces both a hypertrophic and hyperplastic response in many cell types, including renal, cardiac, and vascular smooth muscle cells (VSMCs) (3). The details of the angiotensin II-mediated growth response are emerging. In VSMCs, angiotensin II elicits a hyperplastic response in the presence of serum and a hypertrophic response in serum-free medium (3). Gibbons et al. (40) have shown that angiotensin II induces autocrine production of platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), and transforming growth factor β1 (TGF-β1). PDGF and bFGF are angiogenic factors implicated in a proliferative pathway, whereas TGF-β1 is involved in an antiproliferative pathway (41, 42). In the presence of TGF-β1, the antiproliferative pathway dominates and leads to a hypertrophic response (39–41). Inhibition of TGF-β1 by neutralizing antibodies or antisense oligonucleotides restores a mitogenic response (39, 41). A similar paradigm may apply to renal tissue. Both mitogenesis and hypertrophy have been reported in mesangial cells. Ray et al. (43) have shown that angiotensin II stimulates hyperplasia and fibronectin production in human fetal mesangial cell. The mitogenic response was greater in subconfluent cells and was abolished by administration of an AT_1 receptor antagonist (43). Others have reported a hypertrophic response in rat or murine mesangial cells (44, 45). This response can be converted to hyperplasia by the addition of neutralizing antibodies to TGF-β1 in the culture (44). In rat glomerular endothelial cells and proximal tubular cells, Wolf et al. (46, 47), have also demonstrated a mitogenic response, but noted a hypertrophic response in distal tubular cells (48). Again, the hypertrophic response was conditional on autocrine production of TGF-β1 (46). Taken together, these data indicate that angiotensin II is a bifunctional growth factor that can elicit hypertrophy or hyperplasia depending on individual cell type or the autocrine-paracrine milieu.

Angiotensin II and Fibrosis

Glomerulosclerosis is the final result of many forms of kidney disease and it is characterized by an accumulation of extracellular matrix. Components of the extracellular matrix are secreted by mesangial cells during tissue repair and include collagens, laminin, and fibronectin (49). A maladaptive response to renal injury can lead to excessive deposition of extracellular matrix and result in scarring. As noted above, angiotensin II stimulates renal mesangial and tubular cells to synthesize TGF-β1 and to convert it to its active form. TGF-β1 has emerged as a central autocrine-paracrine factor in the pathogenesis of renal fibrosis (48). It induces the synthesis of extracellular matrix proteins and simultaneously prevents their degradation through the inhibition of proteases and stimulation of protease inhibitors (48). The effects of angiotensin II on extracellular matrix protein production are blocked by AT_1 receptor antagonists or by neutralizing antibodies to TGF-β1 (42, 43). Recent data also suggest that insulin is a hypertrophic factor in mesangial cells (44) and can augment the angiotensin II effects on TGF-β1 and matrix gene expression (50).

Another mediator of angiotensin II action in the kidney is ET-1. Angiotensin I stimulates ET-1 production in cultured renal mesangial and endothelial cells (46, 51). Angiotensin II and ET-1 may have additive effects on cellular growth through hemodynamic and nonhemodynamic pathways. Like angiotensin II, ET-1 has potent vasoconstrictive effects and causes proliferation, TGF-β1 expression, and fibronectin production in renal mesangial cells (3, 51). Studies suggest that it also potentiates the conversion of angiotensin I to angiotensin II through up-regulation of the angiotensin-converting enzyme (52). Hence, angiotensin II and ET-1 may mutually enhance their own production. Gomez-Garre et al. (51) recently have shown that these effects of angiotensin II on mesangial cell growth and fibronectin production can be blocked by an ET-1 antagonist. Conversely, an AT_1 receptor blocker or an ACE inhibitor (51) decreased ET-1-induced changes in mesangial cells. The extent of the interactions between angiotensin II and ET-1 *in vivo* remain to be fully elucidated.

Animal Studies

Interruption of the RAS is renoprotective in many animal models of hypertensive nephrosclerosis. The two-kidney, one-clip Goldblatt model is a classic example of angiotensin II-dependent hypertension and nephrosclerosis (reviewed in ref. 53). The progression of hypertension and glomerulosclerosis is characterized by three stages (reviewed in ref. 53). In the initial phase, the animals develop fulminant hypertension caused by renin activation in the clipped kidney. Plasma angiotensin II levels and renin activity are high and the blood pressure can be normalized by unclipping or using a CEI. The next phase (2 to 4 weeks after clipping) is characterized by maintenance of hypertension despite near normal circulating levels of angiotensin II and suppressed renin expression in the unclipped contralateral kidney (16). This phase is also reversible by unclipping or by using CEIs, demonstrating that the hypertension is still angiotensin II dependent. The angiotensin II content and ACE activity in the contralateral kidney are high and its pressure natriuresis curve is shifted toward higher pressures (14). Thus, investigators have suggested that hypertension is maintained because of either activation of the tissue RAS or a chronic pressor effect of angiotensin II in the contralateral kidney. In addition, renal injury occurs quite early in this model (14). Mai et al. (54) have shown that interstitial inflammation and extracellular matrix deposition occurs by day 7, followed by glomerular damage by day 21 after clipping. This renal injury is progressive and eventually irreversible, leading to a permanent change in the pressure natriuresis curve in the contralateral kidney. Thus, reversal of the stenosis after several months reduces but no longer normalizes blood pressure (third phase) (53). Blood pressure still responds to CEIs at this stage, and long-term treatment with these agents improves survival in the animal, suggesting that tissue RAS may be activated in this phase. Other vasoactive substances participating in the maintenance of hypertension in this model include the products of arachidonic acid metabolism. Himmelstein and Klotman (55) have shown that thromboxane production is markedly elevated in the contralateral unclipped kidney. Administration of thromboxane antagonists restores the pressure-volume regulation and lowers blood pressure (55). Hence, the two-kidney, one-clip Goldblatt model is much more complex than was expected originally. Although there is agreement that the RAS plays a central role in the pathogenesis of hypertension and nephrosclerosis in this model, the role of the local RAS and the exact mechanisms underlying the triphasic blood pressure response remain poorly understood.

A newer model of angiotensin II-dependent hypertension is the transgenic rat harboring the mouse Ren-2 gene [TGR(mREN2)27] (56). These animals develop fulminant hypertension and glomerulosclerosis (56). In this model of hypertensive nephrosclerosis, intrarenal blockade of the RAS restores the pressure-natriuresis relationship to normal (57). In addition, nonhypotensive doses of an AT_1 receptor antagonist have reduced proteinuria and glomerular injury (58). This model is also characterized by near-normal circulating renin levels. Therefore investigators have pointed to the activation of the tissue RAS, particularly in the adrenal glands, as the cause of hypertension (59).

Dahl salt-sensitive rats (Dahl-SS) and spontaneously hypertensive rats (SHR) are two other strains of rats genetically susceptible to hypertension. Dahl-SS rats are unable to regulate preglomerular resistance, and they develop glomerular hypertension and nephrosclerosis quite early (60). Subpressor doses of angiotensin II worsens glomerular hemodynamics and accelerate glomerular injury in these rats (61). In contrast, SHRs can adjust preglomerular resistance and develop renal injury only in old age or after unilateral nephrectomy (62, 63). CEIs normalize glomerular hemodynamics and prevent glomerulosclerosis in aging SHRs with two kidneys (62). In uninephrectomized SHRs, AT_1 receptor antagonists also reduce glomerular damage and reduce TGF-β1 expression (63). The *in utero* treatment of SHRs with CEIs prevents the development of hypertension in the animals and reduces blood pressure in their progeny (64).

Human Studies

Observational studies have shown a direct relation between the level of blood pressure and the development of end-stage renal disease (1, 2, 65). In addition, control of blood pressure slows the deterioration of renal function in essential hypertension or in hypertension caused by kidney disease (66, 67). Based on strong experimental evidence, clinical studies were undertaken to determine whether inhibition of angiotensin II action can have renoprotective benefits beyond its hemodynamic actions. Most of these studies were performed with CEIs because they were the first agents available. The effects of CEIs on the progression of kidney disease have been reviewed recently (68). Treatment with CEIs results in increased renal plasma flow, reduced filtration fraction, and little or no reduction in glomerular filtration rate (68). Protein excretion also diminishes because of a decrease in glomerular hypertension and possibly because of direct blockade of angiotensin II action (68). Studies in humans thus far have shown the renoprotective effects of CEIs in insulin-dependent diabetes, scleroderma, and possibly IgA nephropathy. Scleroderma crisis is characterized by marked activation of the RAS that leads to hypertensive crisis, renal failure, and death (69). The advent of CEIs has markedly improved the prognosis of patients with this disease (70). Studies have also shown that CEIs can reduce the progression of renal failure and proteinuria in immunoglobulin A nephropathy (71). Compelling evidence exists for the use of CEIs in insulin-dependent diabetes; a multicenter study has shown that treatment with captopril is renoprotective independently of the blood pressure level in patients with diabetic nephropathy (72). The use of captopril was associated with reduced proteinuria and a 50% reduction in the combined endpoints of death and need for dialysis or transplantation. In this trial, 75% of the patients were hypertensive, suggesting that the CEIs may also have a role in preventing hypertensive renal injury. A more recent trial compared CEIs with placebo in patients with many renal diseases. It demonstrated that the use of a CEI was beneficial in slowing the deterioration of renal function, independent of blood pressure control (73). However, the rates of the events among the patients with hypertensive nephrosclerosis were too small to allow detection of a beneficial effect specifically within this subgroup. In another study, subjects with hypertension who have normal baseline renal function had a smaller decline in renal function when treated with a CEI than with β-blocker (74). Finally, a recent study has confirmed the beneficial effects of CEIs on the progression of nondiabetic disease (9). Nevertheless, the effect of CEIs in patients with hypertensive nephrosclerosis is still unclear. Studies are currently in progress to determine the efficacy of various antihypertensive regimens on the progression of hypertensive renal disease (75).

Conclusions

In summary, multiple lines of evidence suggest that the intrarenal RAS is a critical neurohormonal factor in the development of renal injury. However, other neurohormonal pathways are also involved in the pathophysiology of the disease. The success of a given therapy in preventing renal diseases may depend on its ability to modulate the circulating as well as the autocrine-paracrine factors promoting renal injury. More research is required therefore to elucidate the interplay among these various factors. Although data indicate that inhibition of the RAS may modify the risk of disease progression in certain forms of renal disease, prospective clinical trials are still necessary to confirm the utility of this approach in hypertensive nephrosclerosis.

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Preventing and Reversing Target Organ Damage by Treatment of the Renin-Angiotensin System

LAWRENCE R. KRAKOFF, M.D., AND VINUTHA RAJASEKHARAIAH, M.D.

FROM 1970 UNTIL THE PRESENT, participation of the renin-angiotensin-aldosterone mechanism in control of the cardiovascular system has become recognized as a pervasive influence on arterial pressure and also as a participant in the pathophysiology of congestive heart failure, ischemic heart disease, and progressive renal insufficiency. Before 1980, the renin system was viewed by many clinicians as an interesting phenomenon to investigate in cardiovascular disorders, particularly hypertension, but with little relevance to treatment despite initial evidence that the patients who have hypertension with normal and high renin levels might be at higher risk for coronary heart disease (1). This suggestion was strengthened subsequently by a prospective study which more clearly linked normal and high renin levels in a hypertensive population with development of myocardial infarction (2).

The activity of the renin system can be measured with reasonable precision through development of radioimmunoassay for its components. However, testing the clinical function of renin-dependent mechanisms awaited the discovery and clinical application of pharmaceuticals whose predominant action is interruption of the renin-angiotensin axis after clues had been provided by studies in experimental animal models. The angiotensin-converting enzyme (ACE) inhibitors, beginning with captopril in 1980, brought to clinical therapeutics a new class of powerful, effective drugs (active when taken orally) to explore the role of the renin system in clinical diseases.

Insights gained from the use of ACE inhibitors have prompted development of the specific angiotensin II, type 1 receptor antagonists as more specific agents for targeting therapy to the presumed active component of the renin system, angiotensin II. This review will focus on the results of strategies for clinical blockade of the renin system in those groups with target organ damage, as it occurs in hypertension, cardiac, vascular, and renal diseases.

Clinical Blockade of the Renin-Angiotensin-Aldosterone System

Several classes of pharmacologic agents inhibit the renin-angiotensin-aldosterone system at various control points. However, the overall action of these drug classes may not be specifically due to their effect on the renin system, because most have other mechanisms of action. Table 1 summarizes the classes of drugs most often used, their site of action on the renin system, and the other mechanisms that must be considered when assessing them. The β-receptor blockers generally reduce secretion of active renin from the juxtaglomerular apparatus by antagonizing the effect of β-receptor agonists such as norepinephrine or epinephrine acting as endogenous adrenergic neurohormonal agents. Many studies have demonstrated that β-receptor blockers reduce plasma renin levels either from baseline or stimulated levels. However, the antihypertensive action of β-

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From the Department of Medicine, Mount Sinai School of Medicine and Englewood Hospital and Medical Center. Address correspondence to Lawrence R. Krakoff, M.D., Professor of Medicine, Mount Sinai School of Medicine, Englewood Hospital and Medical Center, 350 Engle Street, Englewood, NJ 07631.

Key Words: hypertension, target organ damage, reversal of left ventricular hypertrophy

TABLE 1

Actions of the Drug Classes that Antagonize the Renin-Angiotensin-Aldosterone System

Drug class	Effect on the renin-angiotensin-aldosterone system	Other actions
β-receptor blockers	Supression of renin release from the juxtaglomerular apparatus of the afferent arteriole	Direct effects of blockade β-adrenergic receptors: reduced cardiac output, heart rate. Reduced β_2-mediated dilation of blood vessels and bronchi (for nonselective agents).
ACE inhibitors	Reduced conversion of angiotensin I to angiotensin II	Potentiation of bradykinin and related peptides. Stimulation of prostaglandin mediated vasodilation (possibly by bradykinin).
Angiotensin II receptor antagonists Peptide Nonpeptide	Blockade of angiotensin II, type 1 receptors	No other actions known. Peptide antagonists are partial agonists and can raise pressure in low renin states.
Steroid mineralocorticoid receptor antagonists: spironolactone	Blockade of mineralocorticoid receptors of distal renal tubule	Partial blockade of other steroid receptors

receptor blockers is not well correlated with their effect on plasma renin activity levels or with the direct interruption of angiotensin action by an angiotensin-receptor antagonist (3). This lack of correlation is most likely explained by the other actions of β-receptor blockers, especially their direct effect on cardiac β-receptors in reducing adrenergic-induced cardiac contraction and heart rate. The nonselective β-receptor blockers which antagonize both β_1- and β_2-adrenergic receptors impair vasodilation and bronchodilation. In assessing the overall therapeutic effect of the β-receptor blockers, their renin-suppressing effect may be valuable, but other actions probably have greater importance because of the benefit they confer for treatment of hypertension and secondary prevention of coronary heart disease (4).

ACE inhibitors were discovered initially by a nearly serendipitous process when snake venom peptides were found to have kinin-potentiating activity, which prevented the cleavage of the terminal dipeptide of angiotensin I and the formation of angiotensin II. With the subsequent evolution of orally active ACE inhibitors, beginning with captopril, clinical interruption of the renin-angiotensin system became a practical reality. However, the dual action of ACE inhibitors, reduced angiotensin II formation coupled with potentiation of kinin action, has never been eliminated from this class of pharmaceuticals. Thus, the presumption that most of the beneficial effects of AE inhibitors as cardiovascular therapy stem from their action on the renin-angiotensin-aldosterone system remains to be proven. In contrast, the adverse actions of the ACE inhibitors, e.g., cough, seem best related to other mechanisms such as kinin potentiation (5).

The orally active angiotensin II, type 1 receptor antagonists are substituted imidazole, biphenyl compounds, such as losartan, and others are in development and clinical trial (6, 7). These drugs have been shown to be effective for the treatment of hypertension, and recent studies suggest that losartan may also have efficacy for congestive heart failure (8, 9). Proper investigation of the angiotensin II, type 1 receptor antagonists will answer many, but not all questions about the specific role of angiotensin II in the pathogenesis and pathology of hypertension and cardiovascular diseases. The role of angiotensin II, type 2 receptors for cardiovascular regulation and disease has not yet been defined.

Blockade of the mineralocorticoid antagonist by spironolactone has been a part of clinical therapeutics for decades. Spironolactone treatment completely ameliorates both the hypokalemia and hypertension of primary aldosteronism, other mineralocorticoid excess syndromes, and in apparent mineralocorticoid excess caused by 11β-hydroxysteroid dehydrogenase deficiency (10). Spironolactone is a potassium-sparing agent which corrects the hypokalemia of thiazide-type diuretics and, thus, may reduce the likelihood of sudden death during treatment of hypertension (11, 12).

Blockade of the renin-angiotensin-aldosterone system is achieved by a variety of widely used clinical therapeutics. The most recently developed drug class, angiotensin II, type 1 receptor antagonists, now joins older and less specifically targeted agents to establish more completely how antagonism of the renin system may prevent or reverse target organ damage as treatment for hypertension, cardiovascular, and renal diseases.

The next sections summarize the evidence for specific pathologies.

Target Organ Damage and Dysfunction

Left Ventricular Hypertrophy

Before 1990 several reports indicated that treatment of hypertension accompanied by left ventricular hypertrophy (determined by echocardiography) with an ACE inhibitor led to significant reduction of left ventricular mass. Dahlof et al. (13) summarized nearly all these reports in a meta-analysis of various therapies and compared the effects of ACE inhibition with diuretics, β-blockers, and calcium entry antagonists for their effectiveness in reducing left ventricular mass. This summary concluded that the ACE inhibitors were superior to the other classes for diminishing left ventricular mass by the decrements in left ventricular wall thickness. Combining studies of enalapril, captopril, lisinopril, and perindopril, an average reduction in mean arterial pressure of 16 ± 4% (mean ± SD) was accompanied by a diminution in left ventricular mass of 17 ± 9% with 95% confidence intervals of 12% to 22%. Since the publication of that meta-analysis, several other reports and meta-analyses (14) have reached similar conclusions regarding the effects of ACE inhibition.

The relatively smaller effect of calcium channel blockers on reduction of left ventricular mass during treatment of hypertension, noted by Dahlof, may have been biased by dependence on studies using short-acting calcium channel antagonists in comparison with longer acting ACE inhibitors, e.g., lisinopril. Phillips et al. (15) described a reduction in left ventricular mass of 19% after 1 year of treatment of patients with severe hypertension with extended release nifedipine (GITS), in whom a reduction in mean arterial blood pressure of 27% was noted (15). Direct comparisons between different drug classes need to consider pharmacokinetics and duration of action as part of the analysis.

The precise mechanisms whereby ACE inhibition causes reduction in left ventricular mass and left ventricular hypertrophy are not yet certain. Reduction in left ventricular afterload, as systemic pressure decreases, is most important. Other possible favorable actions of ACE inhibition include reduced sympathetic drive to myocardial sites (reflex tachycardia does not accompany ACE inhibition), diminished pre-load resulting from decreased aldosterone secretion with less distal renal tubular sodium reabsorption, and direct effects on myocardial perfusion or growth. The net effect of ACE inhibition on left ventricular systolic function during treatment of hypertension is either a small increase or no change in ejection fraction. Left ventricular diastolic function, i.e., filling patterns, as revealed by radionuclear studies or Doppler echocardiography does not change consistently (16).

Increased left ventricular mass, specifically left ventricular hypertrophy, independently adds risk to high blood pressure for future ischemic heart disease and stroke (17–19). Successful treatment of hypertension should either prevent development of left ventricular hypertrophy or reverse it, if it is present; however, no study has yet established, by the criteria for clinical trials, that treatment of hypertension and reduction of left ventricular mass confers additional benefit. Nonetheless, the rather impressive record of the ACE inhibitors for achieving decreased left ventricular mass during antihypertensive therapy, coupled with evidence that this effect may be largely caused by antagonism of the renin-angiotensin-aldosterone system, should be given major importance in therapeutic strategy.

Congestive Heart Failure

Since publication of the CONSENSUS trial in 1987 comparing enalapril with a placebo (20), many reports, as summarized by Garg et al. (21), have confirmed the beneficial effect of ACE inhibitors for treatment of symptomatic congestive heart failure. In general, use of ACE inhibitors has resulted in decreased overall mortality and death from progressive heart failure. Those with the most severe disease (lowest ejection fractions) seem to have had the greatest benefit, which has been detected within 3 months of onset of treatment. Several ACE inhibitors have been studied for their ameliorative effect on heart failure including captopril, enalapril, lisinopril, ramipril, and quinapril. The overall similarity in pattern of results suggests that ACE inhibition for treatment of heart failure is a class effect.

Is the beneficial effect of ACE inhibition in heart failure caused only by antagonisms of the renin system with no role for the kinin-potentiating action of these drugs? Preliminary studies with the angiotensin II receptor antagonist, losartan, suggest that this drug produces hemodynamic changes similar to those seen with ACE inhibition (22). In a 12-week trial with placebo compared with varying doses of losartan for treatment of heart failure, groups treated with 25 to 50 mg/day had fewer recurrences of heart failure than either the placebo group or patients treated with lower doses of losartan (8). More recently, losartan has been compared with captopril in older patients with heart failure (9). This study was designed and powered only to compare changes in renal function (serum creatinine) between the two treatments. There was no difference in the outcome. However, there were significantly fewer deaths and total hospitalizations in the group given losartan compared with those given captopril. It is then likely that the angiotensin II, type 1 receptor blockers are equal or possibly superior to ACE inhibitors for treatment of congestive heart failure. Thus, evidence from clinical studies supports a conclusion that the effects of ACE inhibition and angiotensin II, type 1 receptor blockade are indeed similar. This similarity is consistent with the hypothesis that blockade of the renin system accounts for most of the beneficial effect of ACE inhibitors in treatment of congestive heart failure. Additional larger trials of the angiotensin II receptor antagonists are necessary for a definitive assessment.

Asymptomatic Left Ventricular Dysfunction

Patients with left ventricular systolic dysfunction, determined by reduced ejection fraction, but without symptoms of congestive failure, nonetheless have a reduced life expectancy and high likelihood of progression to symptomatic heart failure or of sudden cardiac death. They also have been assessed for the benefit of ACE inhibition in several randomized clinical trials. The SOLVD Prevention trial evaluated more than 4000 asymptomatic subjects with ejection fractions ≤35%, comparing a placebo with enalapril (23). Enalapril therapy reduced mortality by only a nonsignificant 12%. However, the ACE inhibitor decreased the combined endpoints of either death or progression to congestive heart failure by 20%, a highly significant effect. In this study, the greatest benefit was in those with ejection fractions <28%, whereas minimal benefit from ACE inhibition was associated with the highest tertile of 33 to 35%.

A surprising outcome of the SOLVD trial was the prevention of myocardial infarction and unstable angina by ACE inhibition in either those with symptomatic heart failure or asymptomatic left ventricular dysfunction (24).

ACE Inhibition After Myocardial Infarction

Nearly 80% of those studied in the SOLVD Prevention trial (23, 24) had ischemic cardiomyopathy or a previous myocardial infarction as the basis for their reduced left ventricular function. These results, and prior experimental studies, suggested that ACE inhibition, e.g., inhibition of the renin-angiontensin-aldosterone system, initiated after an initial myocardial infarction, might retard left ventricular dilation leading to decompensation and, perhaps also prevent ischemic cardiac events (25). Several trials have now been completed and published which generally support that hypothesis.

The SAVE trial was conducted for 4 years and compared placebo with captopril administration initiated several days after an acute myocardial infarction for those with an ejection fraction ≤40% (26). In contrast to placebo administration, the group given the ACE inhibitor had a significant reduction in total mortality (24%), cardiovascular mortality (21%), appearance of congestive heart failure (37%), and recurrent myocardial infarction (25%). In a subgroup analysis, captopril treatment appeared to be more effective for reduction of total mortality in those with ejection fractions ≤32%. However, baseline ejection fraction was not related to the value of captopril treatment for decreasing cardiovascular mortality or morbidity. In a review of the time course of captopril's effect on mortality and morbidity in the SAVE trial, little difference between placebo and active treatment was evident during the first year of observation. During the next 3 years a progressive difference between the two cohorts was revealed. Thus, the delayed effect of ACE inhibitor therapy is consistent with the concept that its mechanism depends on interruption of slowly evolving changes in residual myocardium after the acute infarction, i.e., the "remodeling" hypothesis.

In light of the SAVE study, the results of a short trial (six months) comparing placebo with enalapril administration, started within 24 hours of an acute myocardial infarction (CONSENSUS II), become understandable (27). No difference in mortality was found between the two groups during the observation period of the trial. A nonsignificant trend toward increased mortality in the enalapril-treated cohort was detected during the initial phase of CONSENSUS II when the ACE inhibitor was given very soon after the acute infarction, possibly adding to hypotension. This resulted in a change in design to delay the initial dose of enalapril.

Since 1992, four additional large trials using different designs and different ACE inhibitors have assessed the effect of this drug class on cardiovascular mortality and morbidity after an acute myocardial infarction. The Acute Infarction Ramipril Efficacy (AIRE) trial used ramipril started 3 to 10 days after an acute infarction in patients with "clinical evidence of heart failure" and continued for 1½ years (28). Patients with severe heart failure requiring ACE inhibitors were excluded. After randomization, ACE inhibitor treatment compared with placebo conferred a significant reduction in total mortality (17%), and the combined endpoints of death, severe heart failure, recurrent myocardial infarction, or stroke (19%). A very large multicenter trial conducted at 200 coronary care units in Italy (GISSI-3) compared either lisinopril, nitroglycerine patch, or both with a control group given neither for their effect after an acute myocardial infarction during a 6-week observation period (29). More than 4500 subjects were allocated to each of the four parts of the study. The lowest death rate was observed in the group given both lisinopril and nitroglycerine, 17% less than that of the controls. Nitroglycerine alone had no significant effect on mortality, whereas lisinopril alone had a small (9%) but significant benefit. Subgroup analysis did not disclose that ACE inhibition conferred more specific value for preventing cardiovascular morbidity in this large, but brief trial. The Fourth International Study of Infarct Survival (ISIS-4) (30), a huge, multicenter trial conducted in 58,050 subjects with "suspected myocardial infarction" at 1086 centers worldwide assessed captopril and other therapies. Randomized treatments were given for 1 month, for an observation period of 5 weeks, and a follow-up at 12 months. Captopril treatment for 1 month conferred a small, but significant (7%) reduction in mortality at the 5-week mark, which persisted for 12 months. Extensive subgroup analysis of the study displayed a fairly consistent pattern regarding the beneficial effect of the ACE inhibitor.

In 1995 two more studies were published. The Trandolapril Cardiac Evaluation (TRACE) study evaluated trandolapril compared with placebo in 2606 patients with myocardial infarction, who had an ejection fraction ≤35% by echocardiography (31). Treatment was started 3 to 7 days after the acute infarction, and observations were accumulated during 4 years. Trandolapril or placebo was given for 24 months. Mortality was relatively high in both placebo (42.3%)- and trandolapril (34.7%)-treated groups. However, ACE inhibition was effective by reducing the relative risk of total mortality (22%), cardiovascular mortality (25%), sudden death (24%), and severe heart failure (29%). Based on these results, the number to treat for prevention of a death would be ≈13. Zofenopril was compared with placebo for a 6-week period after an acute myocardial infarction in the Survival of Myocardial Infarction Long-term Evaluation (SMILE) study (32). Assessments at the end of the treatment period and after 1 year disclosed a beneficial effect of the ACE inhibitor for both mortality and the appearance of congestive heart failure.

Does blockade of the renin system explain the overall beneficial effect of the several ACE inhibitors (suggested as a class effect) when given after a myocardial infarction? No information is yet available for the angiotensin II, type 1 receptors to address that issue. Thus, a participating role for kinin potentiation remains to be excluded. The strong trend toward greater benefit of ACE inhibition in those with diminished left ventricular function, i.e., reduced ejection fraction, and during longer periods of observation suggest that the primary effect of this intervention is the result of its effect on evolution of the remodeling process, rather than of a more rapid process involving the coronary arteries themselves. This conclusion is consistent with the studies of ACE inhibition in stable arterial disease described in the next section.

Coronary and Peripheral Atherosclerotic Disease

Angiotensin II is a potent constrictor of the coronary circulation and may have a direct inotropic effect. Diminution of this peptide's action on the coronary circulation might theoretically reduce symptomatic angina and silent ischemia in patients with coronary artery stenosis. Studies of ACE inhibitors have not demonstrated any benefit for stable symptomatic angina, as reflected by either symptoms or exercise tests (33, 34). In contrast, silent ischemic events, as revealed by 48-hour electrocardiographic monitoring, may be reduced by ACE inhibition in patients with proven coronary disease, but good left ventricular function (34). This observation needs to be assessed in long-term studies with clinical outcomes.

A subcategory of the "coronary problem" is the matter of restenosis after angioplasty. Based on experimental studies implying that angiotensin II might participate in proliferation of smooth muscle and fibrous tissue at the sites of endothelial denudation after angioplasty, an ACE inhibitor has been evaluated for its effect on clinical restenosis. Cilazapril was compared with placebo in a double-blind randomized prospective trial (Multicenter European Research Trial with Cilazapril after Angioplasty to prevent Coronary

Obstruction and Restenosis, MERCATOR) (35). Angiographic evaluation and several other indices of coronary disease were assessed 6 months after angioplasty was performed. No significant differences in restenosis rates, anginal occurrences, exercise tests, or clinical endpoints were found in comparing the placebo- and cilazapril-treated cohorts. This study seems to be decisive for excluding ACE inhibition (and perhaps interruption of the renin system) to retard restenosis.

Atherosclerotic stenosis and disease in the carotid arteries account for stroke. The vasoconstrictive effects of angiotensin II and any possible participation in arterial growth imply that reduction in the activity of the renin system might retard progression of atherosclerosis and its ischemic consequences. However, little clinical evidence supports that implication (33). Regarding peripheral arterial disease, i.e., claudication and lower limb ischemia, ACE inhibition may be superior to β-receptor blockade for preservation of limb blood flow and maintaining walking distance (36, 37); however, this effect could as easily be caused by kinin potentiation as to reduction of angiotensin II formation.

Progression of Renal Disease and Proteinuria

Although this topic will be developed in greater detail in Gharavi and Klotman (see p. 118, this issue), an overall assessment of the effect of inhibition of the renin-angiotensin system on target organ damage would be incomplete without mentioning the current state of clinical studies. A decisive clinical trial was conducted in proteinuric diabetic nephropathy for insulin-dependent (IDDM) patients, in which captopril treatment unequivocally retarded progression of renal insufficiency, as measured by either the change in creatinine clearance or requirement for either dialysis or renal transplant (38). Captopril has also prevented the progression of microalbuminuria to proteinuria in IDDM patients (39). ACE inhibition has reduced either microalbuminuria or proteinuria or prevented their appearance in patients with non-insulin-dependent diabetes (NIDDM) (40–42). In nondiabetic renal disease, ACE inhibition may prevent progression, i.e., doubling of serum creatinine, particularly in proteinuric nephropathy and least in polycystic renal disease (43). A recently published meta-analysis also supports the view that ACE inhibition retards the progression of nondiabetic chronic renal disease (44). With regard to the angiotensin receptor antagonists, a limited study of losartan in nondiabetic renal disease with proteinuria reported a reduction in proteinuria (45), suggesting that interruption of the renin system best accounts for the action of ACE inhibitors.

Conclusions

Clinical investigation provides evidence that interruption of the renin-angiotensin system may be beneficial for prevention or reversal of several cardiovascular and renal diseases which are sources of target organ damage both as a result of hypertension and ongoing disease progression. The evidence is based primarily on studies of the ACE inhibitors whose specificity for blocking the renin-angiotensin-aldosterone system is far superior to previously developed therapies, and yet is not without another effect, namely potentiation of kinin action. However, although presently limited, clinical studies of the angiotensin II, type 1 receptor antagonist are consistent with the effects of ACE inhibition. Table 2 summarizes the available results in semiquantitative form to portray this consistency. Several clinical trials are now underway and many smaller studies may be expected to shed more light on this relationship soon (46). The angiotensin II, type 1 receptor has been suggested to account for all the known effects of the renin system on cardiovascular physiology. The characterization of type 2 receptors which bind angiotensin II, the role of which is yet unknown, raises the possibility that ACE inhibi-

TABLE 2

Summary of Effects of Inhibitors of Angiotensin II, Type 1 Receptor Antagonists on Target Organ Damage

Target organ damage	ACE inhibitors	Angiotensin II, type 1 receptor antagonists
Left ventricular hypertrophy	++[a]	?[b]
Congestive heart failure	++	++
Impaired left ventricular function and status postmyocardial infarction	++	?
Stable angina	0[c]	0
Silent ischemia	+	?
Coronary restenosis	0	?
Carotid and peripheral atherosclerosis	0	?
Progression of renal disease-glomerular filtration	++	?
Proteinuria	++	+

[a] Definitive studies from clinical trials.

[b] Unknown or not yet assessed.

[c] No effect, based on available clinical investigations.

tion, decreasing angiotensin II availability at all receptors might differ from selective blockade by losartan-like agents at type 1, but not type 2 receptors. As clinical research evolves, well-designed comparisons between these two drug classes should provide answers and perhaps new questions to explore for a full understanding of how blockade of the renin-angiotensin-aldosterone system contributes to successful therapy.

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Dean's Seminar

Antagonizing Excitotoxicity:

A Therapeutic Strategy for Stroke?

Dennis Choi, M.D., Ph.D.

The development of effective treatments for ischemic stroke has been an elusive goal, but is now coming within our grasp. There are two primary strategies. The first, following the cardiologists' lead, is to limit the ischemic insult itself by lysing the responsible blood clot. Recent data indicate that the thrombolytic agent, tissue plasminogen activator, can improve outcome after ischemic stroke, just as after myocardial infarction, provided that treatment is instituted soon after the onset of symptoms (1). The other fundamental strategy is aimed at the parenchymal level and is driven by the question, "Why is the brain so vulnerable to ischemic injury, anyway?" In modern medicine, it has become almost routine for surgical teams to reattach severed limbs. To my knowledge, however, successful reattachment has not yet been accomplished with the head! The brain is exquisitely sensitive to having its normal steady supply of oxygen and glucose interrupted. If one could understand the basis for this sensitivity and interfere with it—perhaps rendering the brain more like a limb in terms of vulnerability to hypoxia-ischemia—the brain damage induced by a given ischemic insult would be lessened.

Acute thrombolysis and rendering the brain resistant to hypoxia-ischemia are the twin pillars on which our current hopes for effective stroke treatment rest. I will address the latter category, focusing my discussion around the specific idea that glutamate neurotoxicity plays an important role in mediating brain neuronal cell death after hypoxic-ischemic insults (2, 3). This contribution of glutamate neurotoxicity is best considered as a component of a broader concept, the idea that the same specialized cell-cell interactions that allow the brain to do its marvelous job (to think, to process information, to remember) regrettably may convey high vulnerability to damage by hypoxic-ischemic insults. I will also present a personal view of what might be happening in the future in terms of neuroprotective approaches.

Glutamate neurotoxicity, excitotoxicity, was discovered by Lucas and Newhouse (4), and brought into the context of brain neurons by Olney (5). It seems paradoxical that the brain's own workhorse transmitter, glutamate, can become transformed into a neurotoxin. The glutamate hypothesis of hypoxic-ischemic brain damage holds that insults such as hypoxia, hypoglycemia, or ischemia produce rapid cellular energy depletion and depolarization, followed by excessive release of transmitter glutamate through stimulus-secretion coupling or reverse electrogenic transport, compounded by loss of energy-driven uptake systems. These events, increased release and reduced re-uptake, conspire to produce a buildup of glutamate in the extracellular space that easily can be measured in experimental preparations and reach levels high enough to kill neurons (6). A key prediction of the glutamate hypothesis is that antagonizing glutamate toxicity will reduce brain damage in patients presenting with acute strokes.

During the past several years, our understanding of glutamate neurotoxicity has been accelerated enormously by delineation of the molecular biology of glutamate receptors (7–9). There are three major families of glutamate re-

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Address correspondence to Dennis Choi, M.D., Ph.D., Professor and Head, Department of Neurology, Box 8111, Washington University School of Medicine, 600 South Euclid, St. Louis, MO 63110.

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ceptors (10). First, α-amino-3-hydroxy-5-methyl-4-isoxazol propionic acid (AMPA)/kainate receptors mediate the fast synaptic transmissions throughout the central nervous system. These receptors generally open sodium channels, permitting sodium entry and inducing the transient depolarization of the postsynaptic neuron that is fundamental to neuronal signaling. Second, *N*-methyl-D-aspartate (NMDA) receptors gate channels that are not only permeable to sodium, but are also highly permeable to calcium, a property which appears to underlie the prominent involvement of these receptors in synaptic plasticity. Third, at least eight subtypes of metabotropic glutamate receptors are linked via G-proteins to a variety of intracellular processes. Metabotropic glutamate receptors appear to modulate glutamate neurotoxicity, but do not appear to be involved in direct mediation.

The ion channel-linked AMPA/kainate and NMDA receptors mediate glutamate toxicity, although not equally (11). Antagonist experiments *in vitro* suggest that much of the neuronal death associated with brief intense glutamate exposure requires NMDA receptor activation. Glutamate-induced cortical or hippocampal neuronal death can be blocked substantially by selective blockade of NMDA receptors, although predictably (because AMPA/kainate receptors can still be activated), glutamate-induced excitation and acute neuronal swelling are only partly attenuated by the same blockade. In contrast, selective blockade of AMPA/kainate receptors has only a small effect on late neuronal death. Only when both NMDA and AMPA/kainate receptors are blocked is death, as well as acute glutamate-induced neuronal swelling, eliminated.

Although brief exposure to high concentrations of kainate or AMPA produce little death, kainate and AMPA are both highly neurotoxic if exposure is extended for hours. Thus glutamate receptor-mediated neuronal injury, at least on cultured cortical neurons, occurs in two main patterns: rapidly triggered excitotoxicity induced by the brief, intense stimulation of numerous NMDA receptors; and slowly triggered excitotoxicity induced by the prolonged stimulation of AMPA/kainate receptors (or the low-level stimulation of NMDA receptors). Rapidly triggered excitotoxicity depends critically on the presence of extracellular calcium. This is consistent with the idea that rapidly triggered toxicity is initiated by excessive calcium influx through the calcium-permeable NMDA receptor-gated channel. Slowly triggered excitotoxicity may also be initiated by excessive calcium influx. Most channels gated directly by AMPA or kainate receptors have limited calcium permeability. Calcium influx, however, can occur indirectly, for example, through voltage-gated calcium channels activated by the sodium influx and membrane depolarization associated with AMPA/kainate receptor stimulation, or reverse operation of the sodium-calcium exchanger. Most likely, AMPA/kainate receptor-mediated toxicity requires longer periods of receptor stimulation than NMDA receptors, because excess calcium influx takes place more slowly and cellular homeostatic mechanisms are able to compensate better, at least for a while.

The toxic stimulation of glutamate receptors can be considered the induction stage of glutamate-induced neuronal death. Subsequently, events occur that make things worse, promoting further glutamate release and calcium overload. These events can be considered amplification, in parallel to the term used by workers in the synaptic plasticity field. Finally, events occur which actually destroy cells, the expression of glutamate toxicity. It is possible to induce death via toxic overstimulation of glutamate receptors, and then attenuate or prevent death by interfering with amplification or expression events. As a working hypothesis, my colleagues and I envisage that loss of calcium homeostasis, with consequent elevation in intracellular free calcium, is the central link connecting induction to amplification, and also triggers the cascades involved in excitotoxic expression.

Supporting the glutamate hypothesis of hypoxic-ischemic brain damage, substantial evidence now indicates that antagonists of ion channel-linked glutamate receptors can reduce neuronal death in neuronal cell cultures exposed to oxygen-glucose deprivation, or infarct volume in animal models of focal brain ischemia. This is true both for NMDA antagonists (12, 13) and for AMPA/kainate antagonists (14, 15). Glutamate antagonists retain partial neuroprotective efficacy even when administered after the fact, 1 to 3 hours after initiation of ischemia in several animal models. The magnitude of infarct reduction is model dependent, but is typically about 25% to 30% and can exceed 50%. Concern about cytotoxicity slowed drug development for several years, but several glutamate antagonist compounds are either near, or currently in, clinical trial for the acute treatment of stroke. We hope that it will not be too long before we find out whether these drugs, which work well in rodent and even in primate models of stroke, will work in humans.

Given the current burgeoning interest in programmed cell death, I will mention at this point my view that both rapidly and slowly triggered excitotoxic neuronal death preferentially drives neurons toward necrosis rather than toward apoptosis. This view requires an explanation, because all insults, under at least some conditions, probably can trigger programmed cell death leading to apoptosis, and there is every reason to think that this is true of excitotoxicity (see below). However, excitotoxic neuronal death typically is accompanied by early and dramatic cell swelling and relatively little internucleosomal DNA fragmentation (16–18). The gating of numerous membrane glutamate receptor-linked cation channels, with consequent loss of ion homeostasis, effectively may constitute the plasma membrane failure which is at the core of necrosis. Experimentally, my colleagues and I have found that excitotoxic neuronal death in cortical cell cultures, even when induced gradually by low levels of glutamate agonists, remains a necrosis largely insensitive to inhibitors of protein synthesis, to the interleukin-1β-converting enzyme inhibitor, ZVAD, or to deletion of the key apoptosis-promoting gene, *bax* (10).

Before discussing expression events further, I also want to mention an important exception to the rule that AMPA/kainate receptors require lengthy exposures to kill cultured cortical neurons. This exception follows from the recent recognition that some unusual AMPA or kainate receptor subtypes that resemble NMDA receptors in gating channels are highly permeable to calcium (20). In AMPA receptors, this calcium permeability most likely reflects the absence of a key "gatekeeper" AMPA receptor subunit, gluR-2/gluR-B (21, 22), which in its normally edited form conveys the property of calcium impermeability to the AMPA receptor-gated channel. Just as predicted from the idea that excess calcium entry mediates induction of excitotoxic damage, expression of these unusual calcium-permeable AMPA or kainate receptors correlates with enhanced vulnerability to AMPA- or kainate-induced death (23, 24). These unusual calcium channel-gating AMPA or kainate receptors may be expressed preferentially on inhibitory cortical neurons that use γ-aminobutyric acid (GABA) as a primary neurotransmitter (25, 26). The overactivation of these receptors may thus favor destruction of inhibitory neurons, an event which might lead to seizures if excitatory neurons are not similarly depleted. Although seizures are not a common natural complication of ischemic stroke, I speculate that they may surface as a complication if NMDA antagonists are used as neuroprotective agents without other measures aimed at protecting inhibitory neurons from AMPA/kainate receptor-mediated death. Besides the possible contribution of calcium-permeable AMPA or kainate receptors to selective neuronal vulnerability under baseline conditions, recent evidence suggests that ischemic insults selectively may suppress gluR-2/gluR-B expression (27). Thus ischemia may induce alterations in AMPA receptor expression that enhance the subsequent contribution of these receptors to Ca^{2+} influx and cell death.

One potentially attractive future strategy, then, would be to block both NMDA and AMPA/kainate receptors. Not only might this keep inhibitory neurons from being selectively lost; it also might enhance protective effects on the cortical neuronal population as a whole. As I mentioned earlier, NMDA receptor-mediated death dominates in neocortical cultures. Indeed, the addition of selective AMPA/kainate antagonist drugs alone does not produce a detectable increase in the survival of most neurons of oxygen-glucose deprivation; they still die on schedule because of rapidly triggered excitotoxicity. However, if NMDA receptors are blocked first, the addition of AMPA/kainate antagonists produces a substantial incremental protective effect (15). Such combined blockade may have considerable potential for disturbing brain function, but may be acceptable for a short time during a life-threatening illness. Furthermore, improvements in the therapeutic ratio may be obtainable through the use of NMDA or AMPA receptor subtype selective antagonists, although discussion of this is beyond the scope of today's discussion.

Another future direction may be the combination of glutamate receptor antagonists with approaches designed to interfere with downstream events, the expression stage of excitotoxicity. Such a combination may improve the efficacy, or the therapeutic index, of glutamate receptor blockade. Although many deleterious events are likely to occur after loss of cellular calcium homeostasis, three lines of evidence suggest that free radicals may bear heavy responsibility for subsequent death. First, good evidence exists that free radicals are generated in response to glutamate receptor overstimulation, especially of NMDA receptors (28, 29). Second, antioxidant treatments reduce excitotoxic death (30, 31). Last, free radicals by some unknown mechanism can actually potentiate glutamate release, so there is a certain positive feedback potential to this loop (32). Promising antioxidant approaches include the administration of scavenger drugs, or the selective blockade of pathways responsible for generating free radicals after intracellular Ca^{2+} homeostasis is lost, such as lipid breakdown or activation of neuronal nitric oxide synthase. Other important expression events likely include the activation by calcium of proteases and endonucleases.

What happens if one blocks both NMDA and AMPA/kainate receptors in cortical cell cultures exposed to oxygen-glucose deprivation—a paradigm my colleagues and I call "blocked OGD"—and then increases the deprivation period so that neurons still die even though glutamate receptor-mediated death has been eliminated? Under these conditions, two additional injury mechanisms are unmasked. One unmasked mechanism is a modest toxic contribution from voltage-gated sodium and calcium channels (33). Because excessive sodium and calcium influx through agonist-gated channels underlies excitotoxicity, it follows logically that voltage-gated sodium and calcium channels should also contribute to ischemic neuronal death. Their masking by excitotoxicity under native conditions suggests that their contribution to injurious cation influx is small relative to that contributed by glutamate receptors. In fact, measurement of the $^{45}Ca^{2+}$ influx triggered by high potassium-induced membrane depolarization is severalfold smaller than triggered by exposure to NMDA.

Noninactivating voltage-gated sodium channels have been found to play a key role in mediating hypoxic-ischemic damage to the rat optic nerve, a central white matter preparation that lacks glutamate receptors (34). One powerful blocker of such sodium channels is the puffer fish toxin tetrodotoxin, although it is probably unlikely that tetrodotoxin would ever gain approval of the Food and Drug Administration for use in patients! A drug more suitable for clinical use, capable of attenuating current through neuronal noninactivating voltage-gated sodium channels, is the familiar anticonvulsant, phenytoin. Phenytoin has not shown much promise when studied for neuroprotective potential in ischemic stroke, but I speculate that it may become a valuable drug when combined with glutamate antagonist drugs.

A second death mechanism, unmasked when glutamate receptors are blocked and cultured cortical neurons are subjected to prolonged oxygen-glucose deprivation, is apoptosis. Apoptosis plays a vital role in sculpting the nervous system during development, but only in the past few years has evidence accumulated suggesting that it can occur inappropriately in acute and chronic nervous system diseases, including after ischemic insults (35). Neurons exposed to oxygen-glucose deprivation under control conditions develop dramatic cell body swelling comparable with that induced by exogenous administration of glutamate. However, when excitotoxicity is eliminated pharmacologically, prolonged oxygen-glucose deprivation triggers neuronal apoptosis. This is characterized by cell body shrinkage, internucleosomal DNA cleavage, and sensitivity to inhibitors of protein synthesis, to the ZVAD, or to deletion of the *bax* gene.

Cell culture experiments thus suggest that a combination of excitotoxic necrosis and ischemic apoptosis is responsible for neuronal loss after hypoxic-ischemic insults and support a notion that measures specifically directed against each of these mechanisms will find a place in the acute treatment of stroke. Recently, my colleagues and I conducted a preliminary test of this concept by combining the NMDA antagonist, dextrorphan, with the protein synthesis inhibitor, cycloheximide, in a rat model of middle cerebral artery occlusion stroke. Each drug alone, when administered systemically just after the ischemic insult at optimized doses, reduced infarction in the model by about 50%. When combined, nearly an 80% reduction of infarction was obtained (36). Although much further study is needed, dual reduction of necrosis and apoptosis is the most parsimonious explanation for the marked level of neuroprotection obtained in this experiment.

In the foregoing discussion, I have focused on neuronal cell death. However, growing evidence, including this study I just mentioned, suggests that antiexcitotoxic approaches and antiapoptotic approaches reduce brain infarct volume in animal models of stroke. Thus these approaches must reduce the death of glia as well as of neurons. For antiapoptotic approaches, this is not surprising, because some glia death, whatever its etiology, may reflect apoptosis. For antiexcitotoxic approaches, this is harder to understand. However, whereas vulnerability to excitotoxicity classically has been considered to be restricted to neurons, astrocytes (37) and oligodendrocytes (38) can be killed by glutamate receptor overactivation under certain conditions. In addition, some glial death might be triggered by events associated with excitotoxic neuronal death, for example, because of the release of lysosomal contents or cytokine-induced microvascular compromise (39). In the future, it should be possible to delineate why brain glia die consequent to focal ischemia insults and to devise specific glia-preserving countermeasures which might well be combined with approaches for optimizing neuronal cell survival.

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Immunoglobulin and IgG Subclass Levels in the African American and Hispanic Populations of East Harlem

Moshe Lewis, M.D., Michelle Miyashiro, M.D., Jennifer Huton, Lorraine Miller, Ph.D., and Kirk Sperber, M.D.

Abstract

Bacground: The normal levels of immunoglobulin and IgG subclasses in African American and Hispanic populations are uncertain. To determine immunoglobulin and IgG subclass levels in this community, we measured serum IgG, IgM, and IgA levels along with IgG subclasses in 303 African American and Hispanic patients in a general medical clinic and an allergy/asthma clinic in East Harlem in New York City.

Methods: Prospective measurement of immunoglobulins and IgG subclasses in a general medical clinic and an allegy/asthma clinic in East Harlem.

Results: Ten (3.4%) patients had IgG levels below the lower limit of normal values, two (0.07%) patients had IgA levels below the lower limit of normal values, and two (0.07%) patients had an IgM level below the lower limit of normal values. Twenty-four (8.1%) patients had IgG subclass levels below the lower limit of normal values; 1 patient had low levels of IgG_1 and IgG_3, 5 patients had low levels of IgG_2, and 18 patients had low levels of IgG_3. Because low IgG subclasses and allergy/asthma appeared to be associated, we compared IgG subclass levels of the patients with and without allergy/asthma. The mean IgG_2 level in the patients without allergy/asthma was 425.1 ± 199.1 mg/dL ($p = 0.05$) compared with 345.5 ± 133.1 mg/dL in the allergy/asthma group, the mean IgG_3 level in the patients without allergy/asthma was 85.0 ± 57.1 mg/dL compared with 64 ± 34.1 mg/dL in the allergy/asthma group ($p = 0.016$) but there were no differences in IgG_1 and IgG_4 levels between the two groups.

Conclusion: Altogether, our data indicate that humoral immunoglobulin and IgG subclass levels below the lowest normal values occur in the low socioeconomic African American and Hispanic populations, especially in patients with asthma in East Harlem. **Key Words:** Immunoglobulin, IgG subclasses, Hispanics, African Americans.

Humoral immunodeficiencies are a group of diseases characterized by low levels of antibody and recurrent respiratory infections like pneumonia, sinusitis, and bronchitis (1). These heterogeneous disorders can result from various defects in the immune system including abnormalities in B-cell activation of differentiation, T-cell help, T-cell suppression, or accessory cell dysfunction (2). Infections usually are associated with encapsulated organisms, and if the immunodeficiency is left untreated, it can lead to chronic obstructive pulmonary disease and recurrent episodes of sepsis (3–7). Patients with humoral immunodeficiency are also at increased risk of developing autoimmune disease, lymphoma, and gastric carcinoma (1, 8).

The exact prevalence of humoral immunodeficiency in the United States is presently unknown. Most of the studies to determine the incidence of humoral immunodeficiency have been conducted in Caucasian populations in Europe or in Japan and have relied on hospital discharge summaries, hospital pharmacy data, or physician surveys instead of directly measuring immuno-

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Address correspondence to Kirk Sperber, M.D., Division of Clinical Immunology, Mount Sinai School of Medicine, Box 1089, One Gustave Levy Place, New York, NY, 10029.

globulin levels (9–17). Even determining quantitative immunoglobulin levels may not be a true indication of immunoglobulin deficiency because laboratory thresholds for IgG, IgA, IgM, and the IgG subclasses may overlap with a subset of healthy patients. Despite the limitations, however, these studies suggest that there may be ethnic and racial differences in the incidence of humoral immunodeficiency. In a Japanese study the incidence of IgA deficiency was 1 in 16,000, which is significantly lower than in Western countries that display an incidence between 1 in 300 to 1 in 3000 cases (18). There are relatively few studies to date assessing the incidence of humoral immunodeficiency in African American and Hispanic populations in inner cities. These populations have an increased incidence of respiratory tract diseases including asthma (19) which has been associated with humoral immunodeficiency (20–30). To determine the frequency of low levels of immunoglobulins and the IgG subclasses in this community, we measured IgG, IgA, IgM, and IgG subclasses levels in 202 African American and Hispanic patients from East Harlem who were being followed up at the general medical clinic of The Mount Sinai Hospital and 101 age- and ethnically matched patients from the allergy/asthma clinic.

Methods

Patient Populations

General Medical Clinic. In 1994, 202 patients seen at the general medical clinic were selected at random for participation in this study. A serum separator tube of blood for immunoglobulin and IgG subclass determination was obtained after informed consent from patients who were undergoing routine venipuncture. The age, gender, ethnic background, country of origin, address, diagnosis, and a chart review were obtained for each of the patients.

Allergy/Asthma Clinic. In 1994, 101 patients seen at the Adult Allergy/Asthma Clinic of The Mount Sinai Hospital were also included in this study. The patients were selected for entry into the study if they had a diagnosis of asthma for longer than 6 months, had positive skin tests to a panel of aeroallergens including ragweed, cocklebur, lamb's quarters, mixed trees, mixed molds, dog, cat, cockroach, house dust and dust mite, were older than 16 years of age, and had no history of immunodeficiency. If the patients met these criteria, the following clinical data were obtained: age, gender, ethnic background, country of origin, duration of asthma, hospitalizations for asthma during the preceding 5 years, immunotherapy status (IT), mean annual rate of sinusitis, bronchitis, pneumonia, viral respiratory tract infections, and allergy skin test results. An aliquot of serum was cryopreserved for determination of immunoglobulin and IgG subclass. No patient with a history of HIV-1 infection or risk factors for HIV-1 infection was included in either the general medical or allergy/asthma clinic populations.

Laboratory Assessment

Quantitative immunoglobulin measurements for IgG, IgM, and IgA were determined by a rate nephelometer using a commercially avaiable kit (Pharmacia, Piscataway, NJ). The manufacturer's reference range based on the manufacturer's controls was established from a population of 200 apparently healthy men and women from California, including Caucasian and Asian persons. Normal values also were established from controls in our laboratory. The IgG subclasses were measured using commercial kits (The Binding Site, San Diego, CA). The manufacturer's reference values were established from 124 healthy adult British blood donors, and normal values were established from controls in our laboratory. We determined a normal reference range in 106 age-matched (48.7 ± 23 years), gender-matched (71 females, 35 males), and ethnically matched (69 Puerto Ricans, 37 African Americans) controls. This reference range did not differ significantly from the manufacturer's reference for immunoglobulins or the IgG subclasses. When subclass levels were high, determinations on the original serum were repeated after appropriate dilution using monoclonal antisera-coated plates for the individual subclasses. When levels were low, determinations of the original serum were repeated using low-range polyclonal antisera plates specific for each subclass.

Statistical Analysis

Statistical analysis was performed using previously established methods. The data were transformed into natural logarithms to avoid undue influence of outlying values. Data were compared by χ^2 analysis, and mean values were compared by a two-tailed Student's *t*-test. A *p* value of 0.05 was established as the criterion for statistical significance (31).

Results

The population used in this study was recruited from the general medical and allergy/asthma clinics at The Mount Sinai Hospital and reflects the demographics of the community that surrounds the hospital. Two hundred and two general medical patients and 101 allergy/asthma patients met the eligibility criteria for entry into the study (Table 1). There were 76 men and 227 women. Of the 303 patients studied, 69 were African Americans, 233 were Hispanic, and 1 was Native American; 114 patients had hypertension, 50 had coronary artery disease, 25 had diabetes mellitus, 7 had rheumatoid arthritis, 5 had sarcoidosis, 29 had asthma alone, 13 had allergic rhinitis alone, and 66 had both asthma and allergic rhinitis. Sixty patients with asthma required multiple medications for control of their disease including 30 who required oral steroids (20 controlled with prednisone ranging from 10 to 40 mg daily and 10 controlled with methylprednisolone ranging from 8 to 24 mg daily). The patients were highly allergic; house dust, dust mites, dogs, cats, and cockraoches were the five most common allergens. The patients were assigned to three groups by diagnosis: general medical disorders, allergy and asthma, and inflammatory diseases including rheumatoid arthritis. Age, steroid usage, immunoglobulin, and IgG subclass levels are also included (Table 1).

Incidence of Immunoglobulin Levels below The Lowest Normal Values

Because quantitative immunoglobulin levels were not normally distributed, natural logarithmic values were used: The quantitative immunoglobulin levels are shown in Table 2 for the general medical clinic and allergy/asthma groups of patients; the distribution of the range of values is illustrated in Fig. 1. As a group, the 202 patients from the general medical clinic had mean antibody levels that were within normal limits. However, 10 (3.4%) patients had immunoglobulin levels below the normal range using an age- and ethnically matched control group for normal ranges (IgG, 750–1702 mg/dL; IgA, 94–398 mg/dL; IgM, 48–250 mg/dL; see Table 3). Of these 10 patients, 9 were female and 1 was male. Six patients had low IgG levels, two patients had low IgA levels, and two had low IgM levels. Seven patients were Hispanic, two were African American, and one was Native American. The mean age of the patients was 58.2 ± 13.7 years. Of the patients that we identified with low antibody levels, nine had either chronic lung diseases including

TABLE 1

Patients Grouped by Diagnosis

A. General medical disorders (189)

Demographics

Mexican	Colombian	Puerto Rican	Native American	African American	Caucasian	Age (yr)	Male	Female	Oral steroids
4	6	122	1	46	0	53.3	63	126	None

Immunoglobulins and IgG Subclasses (mg/dL)

IgG	IgA	IgM	IgG_1	IgG_2	IgG_3	IgG_4
1250.9 ± 391	325 ± 127.1	148 ± 127	654 ± 275	370.4 ± 180	89.1 ± 57.1	47.1 ± 35.1

B. Allergy/asthma (112)

Demographics

Mexican	Colombian	Puerto Rican	Native American	African American	Caucasian	Age (yr)	Male	Female	Oral steroids
1	1	94	0	20	0	52.1	41	67	Yes

Immunoglobulins and IgG Subclasses (mg/dL)

IgG	IgA	IgM	IgG_1	IgG_2	IgG_3	IgG_4
1277 ± 388	344 ± 144	155 ± 122	633 ± 233	340.4 ± 166	64 ± 32	42.2 ± 32

C. Inflammatory diseases (12)

Demographics

Mexican	Colombian	Puerto Rican	Native American	African American	Caucasian	Age (yr)	Male	Female	Oral steroids
0	0	9	0	3	0	50.1	3	9	None

Immunoglobulin and IgG Subclasses (mg/dL)

IgG	IgA	IgM	IgG_1	IgG_2	IgG_3	IgG_4
1199 ± 267	302 ± 145	137 ± 109	624 ± 277	323 ± 145	79 ± 49.1	39 ± 26.1

TABLE 2

Immunoglobulin and IgG Subclass Levels

Antibody	Non-asthma (mg/dL)	Allergy/asthma (mg/dL)	Reference range
IgG	1231.1 ± 345.1	1224.1 ± 332.1	1201 (750–1702)*
IgA	325 ± 175.0	323 ± 78.1	275 (94–398)*
IgM	156.6 ± 13	153 ± 143	140.5 (48–250)*
IgG_1	654 ± 257.2	660 ± 211.1	610 (352–977)*
IgG_2	425.1 ± 199	345.5 ± 133.1 ($p = 0.05$)	348 (120–548)*
IgG_3	85.0 ± 57.1	64.1 ± 34.1 ($p = 0.016$)	63.5 (37–127)*
IgG_4	45.1 ± 34.4	46.1 ± 35.1	30.0 (1–141)*

* 95% confidence limit using a control group consisting of 106 normal healthy ethnically and age-matched controls for the immunoglobulins and IgG subclasses. Normal ranges of immunoglobulins and IgG subclasses were determined in our laboratory.

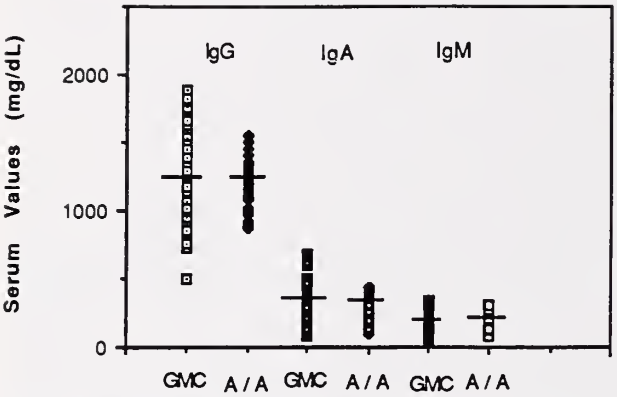

Fig. 1. Scatter plot of serum levels of IgG, IgA, and IgM in patients seen in the general medical (GMC) and allergy/asthma clinics (A/A).

allergic rhinitis and asthma, whereas three had arthritis, two had autoimmune diseases, two had gastrointestinal diseases, and two had skin infections. All the patients had multiple diseases, and none were taking medications that would affect immunoglobulin levels (Table 3). In the allergy/asthma clinic population, IgG levels were affected by oral steroid therapy. There was a statistically significant reduction in IgG for both short-term (<6 weeks) (1286.4 ± 625 mg/dL vs 980 ± 451 mg/dL, $p = 0.001$) and long-term (> 6 weeks) (1301 ± 721 mg/dL vs 1103.1 ± 525 mg/dL, $p = 0.01$) steroid use. The immunoglobulin levels were not altered by inhaled steroids. No patient in the allergy/asthma clinic population had low IgA or IgM levels (data not shown).

Incidence of IgG Subclass Deficiency

Because 9 of the 10 patients identified with low immunoglobulin levels had chronic respiratory diseases (Table 3), we wanted to determine the prevalence of IgG subclass levels below lowest normal values which is associated with recurrent respiratory infections. We identified 24 (8.1%) patients who had levels below lowest normal values in one or more IgG subclasses (10 low IgG_3, 2 low IgG_2, and 1 IgG_1- and IgG_3-deficient patients, see Table 4) in the general medical clinics population and in 11 patients (8 IgG_3 deficient and 3 IgG_2 deficient) in the allergy/asthma clinic population (Table 5) using an age- and ethnically matched control group (IgG_1, 352–977 mg/dL; IgG_2, 126–548 mg/dL; IgG_3, 37–127 mg/dL; IgG_4, 1–141 mg/dL). The distribution of the IgG subclass range of values is shown in Fig. 2. The mean age of the patients with low subclasses was 58.7 ± 21.4 years (16 females and 8 males). Similar to the patients with low total immunoglobulin levels in the general medical clinic group, 10 of the 13 patients with low IgG subclasses had either allergic rhinitis or asthma, 1 had rheumatoid arthritis, and 2 had autoimmune diseases. Twelve (12%) of 101 allergy/asthma clinic patients had low IgG subclass levels, including five patients taking oral steroids. Patients with low IgG_3 subclass levels had more frequent episodes of sinusitis. There was no relationship between immunoglobulin or IgG subclass levels and immunotherapy status or degree of atopy as determined by allergy skin testing (data not shown). Similar to the quantitative immunoglobulins, IgG subclass levels were also reduced in patients taking oral steroids compared with those patients not taking oral steroids. The reduction was statistically significant for IgG_1 (650.9 ± 321 mg/dL vs 509.2 ± 251 mg/dL, $p = 0.03$) and trending toward significance for IgG_2, IgG_3, and IgG_4.

Comparison of Immunoglobulin and IgG Subclass Levels Between Non-allergy/Asthmatic Patients and Allergy/Asthma Patients

Because most of the patients with low total IgG and IgG subclasses had either allergic rhinitis or asthma we compared immunoglobulin and IgG subclass levels of the general medical clinic group with those of the allergy/asthma clinic

TABLE 3

Correlation of Low Immunoglobulin Levels with Clinical Disorders[a]

Pateint no.	Sex	Age (yr)	IgG (750–1702)	IgA (94–398)	IgM (48–250)	Clinical manifestation
1	F	43	745 (L)	135	215	Asthma/allergery
2	M	56	505 (L)	170	330	Chronic bronchitis, RA
3	F	78	785	75 (L)	50	Asthma, arthritis
4	F	68	930	1760	35 (L)	Asthma, arthritis
5	F	63	730 (L)	120	105	Asthma, Crohn's
6	F	72	840	75 (L)	115	Arthritis, skin infections
7	F	70	1000	240	35 (L)	Asthma, skin infections, RA
8	F	40	720 (L)	131	58	Asthma
9	F	43	700 (L)	200	215	Asthma
10	F	44	674 (L)	300	49	Asthma

[a] RA, rheumatoid arthritis. Values in parentheses represent normal range (mg/dL). None of the patients received or were receiving corticosteroids. (L) = low.

TABLE 4

Correlation of Low IgG Subclass Levels with Clinical Disorders in the Patients Attending the General Medical Clinic[a]

Patient #	Sex	Age (yr)	IgG (mg/dL)	IgG_1 (mg/dl)	IgG_2 (mg/dL)	IgG_3 (mg/dL)	IgG_4 (mg/dl)	Clinical manifestation[b]
1	F	54	906	541	244	27 (L)	26	Asthma
2	F	64	1002	826	206	28 (L)	66	Asthma
3	F	59	770	311 (L)	312	26 (L)	10	Asthma
4	F	52	877	573	312	26 (L)	10	Asthma
5	F	58	979	495	196	32 (L)	10	Asthma
6	F	59	1009	876	105 (L)	161	34	Asthma
7	M	65	1344	698	566	19 (L)	46	Asthma
8	F	54	1248	722	124	38 (L)	47	Asthma
9	F	58	1033	768	124	12 (L)	30	Asthma
10	M	59	1123	541	511	29 (L)	13	Allergy
11	F	58	1677	1558	106 (L)	89	29	RA
12	F	64	809	408	266	9 (L)	60	SLE
13	M	69	834	587	194	27 (L)	32	SLE

[a] See Table 3 for normal range for each IgG subclass. None of these patients received or were receiving corticosteroids.

[b] SLE, systemic lupus erythematosis; RA, rheumatoid arthritis. (L) = low.

TABLE 5

Correlation of Low IgG Subclasses with Clinical Disorders in the Allergy/Asthma Clinic Patients[a]

Patient no.	Sex	Age	IgG (mg/dL)	IgG_1 (mg/dl)	IgG_2 (mg/dL)	IgG_3 (mg/dL)	IgG_4 (mg/dl)	Clinical manifestation	CS
14	F	48	799	456	90 (L)	46	67	AR/asthma	No
15	F	57	887	567	85 (L)	67	43	AR/asthma	No
16	F	54	909	658	36.5 (L)	39	32	AR/asthma	Yes
17	F	53	866	488	115	32.3 (L)	6	AR/asthma	No
18	F	52	904	504	134	31.0 (L)	19	AR/asthma	No
19	M	51	1008	688	151	28.6 (L)	13	AR/asthma	No
20	M	67	799	422	176	27.5 (L)	56	AR/asthma	Yes
21	M	48	877	522	188	27 (L)	44	AR/asthma	No
22	F	40	799	466	201	25 (L)	33	AR/asthma	No
23	F	40	900	498	243	22 (L)	67	AR/asthma	No
24	F	49	1008	599	224	15 (L)	55	AR/asthma	Yes

[a] See Table 3 for normal range values of each IgG subclass. AR, allergic rhinitis; CS, corticosteroids. (L) = low.

group. Because steroid therapy reduced total IgG and IgG_1 subclass levels we also excluded the 22 patients taking oral steroids from both the general medical and allergy/asthma clinics. There were no differences in IgG, IgM, and IgA levels between the two groups, but there were significantly lower IgG_2 (425.1 ± 199 mg/dL vs 354.5 ± 133.1 mg/dL, $p = 0.05$) and IgG_3 (85.0 ± 57.1 mg/dL vs 64.1 ± 34.1 mg/dL, $p = 0.016$) levels in the allergic rhinitis and asthma patients compared

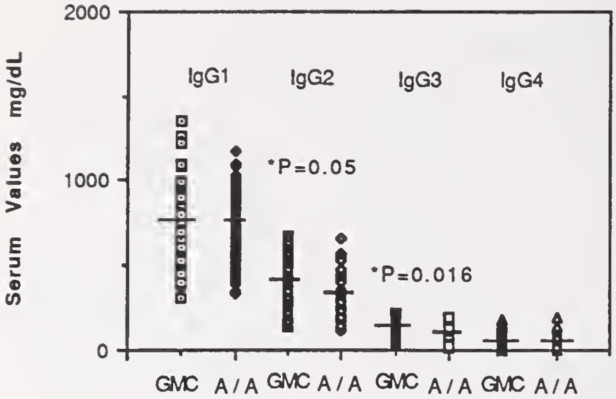

Fig. 2. Scatter plot of the serum levels of IgG subclasses in patients seen in the general medical (GMC) and allergy/asthma clinics (A/A). The IgG$_2$ and IgG subclasses were lower in the A/A group than the general medical clinic group (GMC).

with the non-allergic rhinitis/asthma group (Table 2).

Discussion

Most of the studies to date determining the frequency of humoral immunodeficiency have focused on either European or oriental populations (9–18). There are relatively few data available investigating the frequency of humoral immunodeficiency in Hispanic and African American populations. These populations generally do not have good access to medical care. This study only sought to determine the frequency of low levels of immunoglobulin and IgG subclasses in the Hispanic and African American community in East Harlem. To more fully evaluate immunodeficiency, patients with low immunoglobulin and IgG subclass levels should be evaluated functionally by immunization with different vaccines, assessing pre- and postimmunization titers. Because HIV-1 infection is prevalent in East Harlem and infection can alter immunoglobulin levels (typically increasing them), we excluded known risk groups from this study. Owing to the constraints of HIV-1 testing, it was not possible to test all the patients in this study for HIV-1.

Respiratory diseases including asthma are very prevalent in this patient population and are associated with a high morbidity and mortality (19). Our sample population from the general medical clinic which included 73.5% Hispanics and 25.5% African Americans is representative of the African American and Hispanic community of East Harlem. Of the 202 patients, 102 were from Puerto Rico and had lived in the United States an average of 27 years. Although ethnically representing the East Harlem community, the clinic population was older than the general population (mean age, 68 years) and were being followed up for chronic illness including hypertension, diabetes, and asthma. Of the 303 patients that were screened in the Internal Medical Associates' offices (IMAs) and allergy/asthma clinics, 10 (3.3%) patients had low antibody levels and 24 (7.0%) had low IgG subclass levels. Nine of the 10 patients with low antibody levels and 18 of the 24 patients with low subclasses were women. Although this is not surprising because the clinic study population was predominantly female, immunoglobulin deficiency may be more prevalent in women. Of the 103 antibody-deficient patients reported by Cunningham-Rundles (1) 65% were female. Gender may also influence outcome, because women with antibody deficiency live longer (average age at death, 55 years) than men (average age at death, 29 years) (1). Of the patients identified with antibody levels below lowest normal values, seven were Hispanic of Puerto Rican descent (70%), two were African American (20%), and one (10%) was Native American. We attempted to define ethnicity by defining the patient population on the basis of country of origin, but this may not be completely adequate.

The spectrum of disease that the antibody-deficient patients experienced was similar to what has been reported in Caucasian and Oriental populations including respiratory, gastrointestinal, and autoimmune diseases (1, 3–8, 32). One of the most intriguing findings in the general medical clinic study population was the association of asthma with low IgG$_2$ or IgG$_3$ levels. In a recent study, Parma-Carlos and Parma-Carlos (33) identified 37 patients with primary immunodeficiency in 5100 out-patients followed up in an allergy/immunology clinic. There appeared to be a potential association of IgG subclass deficiency and asthma (26, 27). For this reason, we also analyzed a similar demographic population in the allergy/asthma clinic. We identified 11 other patients with low IgG subclasses. Recent studies have noted the association of IgG subclass deficiency with upper and lower respiratory tract infections and asthma (20–30). Most of these studies demonstrated IgG$_2$ and IgG$_3$ deficiency in preselected populations that were not atopic. To determine whether there was an association between lower subclass levels and asthma in our study population, we compared IgG$_2$ and IgG$_3$ subclass levels in the asthmatic and nonasthmatic patients. There were significantly lower levels of IgG$_2$ (425.1 ± 199 mg/dL vs 354.5 ± 133 mg/dL, $p = 0.05$) and IgG$_3$ (85 ± 57.1 mg/dL vs 64.1 ± 34.1 mg/dL, $p = 0.016$) in the asthmatic group than the nonasthmatic group. Although the relationship between the low IgG subclass levels and asthma is not obvious, lower levels of these two subclasses may contribute to increased infections and exacerbate the disease. In some studies, lower IgG_3 levels have been associated with an increased incidence of sinusitis and obstructive lung disease (22, 32, 33).

In conclusion, immunoglobulin and IgG subclass levels below the lowest normal values occur in the low socioeconomic African American and Hispanic populations in East Harlem. Patients with decreased immunoglobulin and IgG subclass levels have respiratory disease, especially asthma, arthritis, and autoimmune diseases. IgG_2 and IgG_3 subclass levels were lower in the asthmatic group than nonasthmatic group. Larger studies may be needed to better ascertain the true incidence of humoral immunodeficiency in the African American and Hispanic community.

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Amaranthin Lectin Binding in the Rat Colon:

Response to Dietary Manipulation

EVREN O. ATILLASOY, M.D.,[1] ANARGYROS KAPETANAKIS, M.D.,[1] STEVEN H. ITZKOWITZ, M.D.,[2] AND PETER R. HOLT, M.D.[1]

Abstract

Background: In human colon, binding of the lectin *Amaranthus caudatus* has been considered to be a marker of cellular proliferation and malignant progression. We studied regional amaranthin binding in rat colon and correlated this with physiologic manipulations of proliferation.

Methods: Binding of amaranthin in segments of proximal and distal colon was studied in starved, refed, and control Wistar rats and was compared to tritiated thymidine labeling and proliferating cell nuclear-antigen expression.

Results: Amaranthin bound mainly to cells in the lower crypt of distal colon and midcrypt of proximal colon, paralleling the distribution of proliferative markers. Binding occurred in the supranuclear region in distal colon and the pericellular membrane in proximal colon. Starvation/refeeding was associated with a change in amaranthin binding intensity in distal colon, but not in proximal colon.

Conclusion: The pattern of amaranthin binding during starvation/refeeding seems to reflect physiologic changes in several areas of the colon. **Key Words:** amaranthin, colon, proliferation, lectin binding, starvation, refeeding.

IN HUMAN AND RODENT LARGE INTESTINE, the proximal colon (PC) and distal colon (DC) show distinctive histologic features which may relate to differences in functions, carcinogenic propensity, and rates of replication of crypt epithelium. In particular, the proliferative zone in the PC is in the midcrypt, whereas in the DC it is in the lower crypt (1). The expression of cellular glycoconjugates detected by lectin binding also has shown regional differences. Lectins, as carbohydrate-specific probes, are used primarily to assess how glycoconjugate expression relates to the processes of cell differentiation and malignant transformation in the colon (2). Less is known about whether lectin binding reflects changes in cell proliferation and almost nothing is known about the physiologic regulation of cellular glycoconjugates by dietary, luminal, or neurohumoral factors.

The lectin, *Amaranthus caudatus* (ACA), binds specifically to the terminal sugars of the putative carcinoma antigen, the Thomsen Friedenreich (or T) antigen, and its sialylated variants (3). In normal human colon, distinct regional patterns of ACA reactivity have been observed. In a study that primarily examined specimens from the distal colon, the binding of ACA to cells in the lower crypt led to the conclusion that ACA may be a marker of the crypt proliferative zone (4). Other investigators have disputed the

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From the [1]Division of Gastroenterology, St. Luke's-Roosevelt Hospital Center and the Department of Medicine, College of Physicians and Surgeons of Columbia University, New York, NY and the [2]Division of Gastroenterology, Department of Medicine, Mount Sinai School of Medicine, New York, NY. Address correspondence to Peter R. Holt, M.D., Chief, Division of Gastroenterology, St. Luke's-Roosevelt Hospital Center, 1111 Amsterdam Avenue, New York, NY 10025.

role of ACA as a proliferative marker since ACA binding did not correspond to proliferating crypt cells in the human proximal colon (5).

Although ACA reactivity is described in the fetal rat colon (6), little is known about ACA reactivity in the adult rat colon and whether this might change in response to physiologic stimuli. Our previous studies (7, 8) of colonocyte proliferation in the rat used a starvation-refeeding model which can reproducibly down-regulate and up-regulate proliferation. In the present study, we describe ACA reactivity in the normal adult rat colon and changes that occur in response to starvation/refeeding. ACA reactivity is compared with known markers of cellular proliferation including proliferating cell nuclear antigen (PCNA) and tritiated thymidine (^{3}HdThd).

Materials and Methods

Starvation-Refeeding

Six-week-old male Wistar rats, weighing approximately 180 g, were obtained from Harlan Industries (Indianapolis, IN). The rats were individually housed in our animal-care facility in a temperature-controlled and dark-light cycled (1800–0600 dark) room to control the diurnal cycle of intestinal cell production. The animals received proper care and maintenance in accordance with institutional guidelines. The animals were allowed to stabilize their body weights for at least 8 days before the start of the study.

Three groups of four animals each were used. The control group consisted of rats fed *ad libitum* and studied on the morning of the experiment. The second and third groups were starved for 3 days but the second group (starved) was allowed water *ad libitum;* the third group (refed) was allowed water and chow *ad libitum* for 24 hours before being studied. Food intake of the third group was similar to that found in previous studies (7). One hour before being killed, rats in each group were injected with 400 μCi/kg of ^{3}HdThd intraperitoneally.

The animals were killed between 0900 and 1000 hours. One-centimeter segments from the middle ascending colon [designated proximal colon (PC)] and the middle descending colon [designated distal colon (DC)] were removed, fixed in 10% formalin, then immersed in 70% alcohol for 4 hours. After that, the segments were placed in 95% alcohol and embedded in paraffin. The sections were oriented carefully to evaluate biotinylated ACA, PCNA, and ^{3}H labeling of the epithelial cells longitudinally from the crypt base to the surface.

Lectin Histochemistry

The formalin-fixed paraffin-embedded sections were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked by incubation in 3% H_2O_2 in methanol for 20 minutes. The slides were washed and then immersed in phosphate-buffered saline (PBS)/1% bovine serum albumin (Sigma, St. Louis, MO). Sections were then incubated with biotinylated ACA (Vector Laboratories, Burlingame, CA) at a concentration of 1.5 μg/mL in PBS. After washing, the slides were incubated with 6 μg/mL streptavidin-peroxidase conjugate (Zymed Laboratories, San Francisco, CA) for 30 minutes. After washing, the sections were incubated in a freshly prepared 1% diaminobenzidine (DAB) (Sigma, St. Louis, MO) mixture for 10 minutes, counterstained with methyl green, dehydrated, and mounted.

Autoradiography

Four-micrometer sections from DC and PC were coated with NTB2 emulsion (Eastman-Kodak, Rochester, NY). A 2- to 4-week period of exposure at 4°C was followed by 3 minutes of development in Kodak D-19, and 2 minutes of fixation in Rapidfix. The slides were then stained with hematoxylin and eosin, and mounted in Permount (Fisher Scientific, Fairlawn, NJ).

PCNA Immunohistochemistry

Alcohol-fixed DC sections were deparaffinized and immersed in 3% H_2O_2 for 5 minutes. The tissue was incubated with blocking serum for 20 minutes to reduce background staining. The sections were incubated for 180 minutes with monoclonal mouse IgM anti-PCNA antibody (Coulter Immunology, Hialeah, FL) at a working concentration of 1.2 μg/mL. The slides were then incubated for 45 minutes with biotinylated goat anti-mouse immunoglobulin (Zymed Laboratories, San Francisco, CA), diluted 1:2000, and then incubated with avidin-biotin-horseradish peroxidase complex (Vectastain ABC kit; Vector Laboratories, Burlingame, CA) for 30 minutes. The tissue sections were then treated for 10 minutes with 0.1% DAB (Sigma, St. Louis, MO) in PBS to which 1.7 mL of 30% H_2O_2/L was added before use. The slides were counterstained with 2.5% hematoxylin for 60 seconds, dehydrated, and mounted in Permount. Between incubations, the slides were washed three times with PBS. All incubations were performed in a humidified chamber at room temperature.

Scoring of ACA Staining

Cellular staining patterns in cytoplasm and of the apical and lateral membranes were defined using a light microscope at a magnification of ×250 and ×400. ACA staining was evaluated separately for intensity of staining and for distribution within cells along the crypt. Scoring of both immunohistochemical and autoradiographic results was performed in a blinded fashion by one observer, without knowledge of the colonic location or the feeding status of the animal.

The crypt was divided into four compartments: the lower third, the middle third, the upper third, and the crypt surface. The intensity of staining within each compartment was scored on a scale of 0 (no staining) to 4 (maximal staining). For each crypt compartment, the percentage of ACA-positive cells was designated as follows: (+) 1% to 25%; (++) 26% to 50%; (+++) 51% to 75%. A minimum of 15 crypts per segment per animal was used for analysis. The intensity and distribution of the reaction product were expressed separately for each of the study conditions by compartment.

Scoring of ^{3}HdThd Nuclear-Labeled and PCNA-Positive Colonocytes

A cell was judged to be labeled by ^{3}HdThd autoradiography if five or more black silver grains were seen overlying the nucleus. A cell was scored as PCNA positive if the nucleus was stained brown after immunoperoxidase staining. Longitudinally sectioned crypts were scored only if colonic epithelial cells were visible along a crypt column from the base of the crypt to the crypt-surface junction on either side or both sides of a crypt. The cells were counted starting from the base of the crypt and ending at a cell located on the crypt surface midway between the mouths of two adjacent crypts. The number of labeled or immunoreactive cells, their position along the crypt column, and the total crypt cell length were recorded. The crypt column was subdivided into three compartments: the lower third, the middle third, and the upper third. Labeling indices for each compartment were determined by dividing the number of labeled or PCNA-positive cells by the total cell number in that compartment. A minimum of 15 to 20 crypt columns were counted per rat in each colonic segment.

Statistical Analysis

Differences in binding intensity of ACA lectin and labeling indices of proliferation markers were analyzed by paired t testing.

Results

ACA Binding Intensity and Crypt Cell Distribution

Table 1 depicts the binding of ACA to cells in the PC and the DC. In the DC, in control conditions, colonocytes bound ACA with greatest inten-

TABLE 1
Crypt Cell Distribution of ACA Staining[a]

	Binding					
	Group 1 Control		Group 2 Starved		Group 3 Refed	
	Intensity	Frequency	Intensity	Frequency	Intensity	Frequency
Crypts in distal colon						
Surface	2.00 (0.41)[b]	++	1.75 (0.33)	++	2.00 (0.25)	++
Upper third	1.75 (0.48)	+	0.50 (0.33)[c]	+	1.75 (0.25)	++
Middle third	2.25 (0.25)	++	0.50 (0.33)[d]	++	2.25 (0.41)	++
Lower third	3.25 (0.48)[b]	+++	1.25 (0.00)[d]	+++	2.75 (0.25)	++
Crypts in proximal colon						
Surface	1.50 (0.29)	++	1.25 (0.63)	++	2.00 (0.29)	++
Upper third	0.75 (0.48)	+	1.00 (0.41)	+	1.33 (0.50)	++
Middle third	1.75 (0.48)[e]	+++	2.25 (0.25)	++	2.70 (0.50)[e]	++
Lower third	0.50 (0.29)[e]	+	1.25 (0.48)	+	0.70 (0.50)[e]	+

[a] Data from examination of a minimum of 15 crypts per rat ($n = 4$; $n = 3$ for proximal colon refed). Mean binding intensity ± SEM (in parentheses) is based upon an arbitrary scale where 0 = no binding and 4 = maximal binding intensity.

[b] Data designated in DC are significantly different from each other; $p < 0.05$.

[c] $p < 0.05$ compared with control rats.

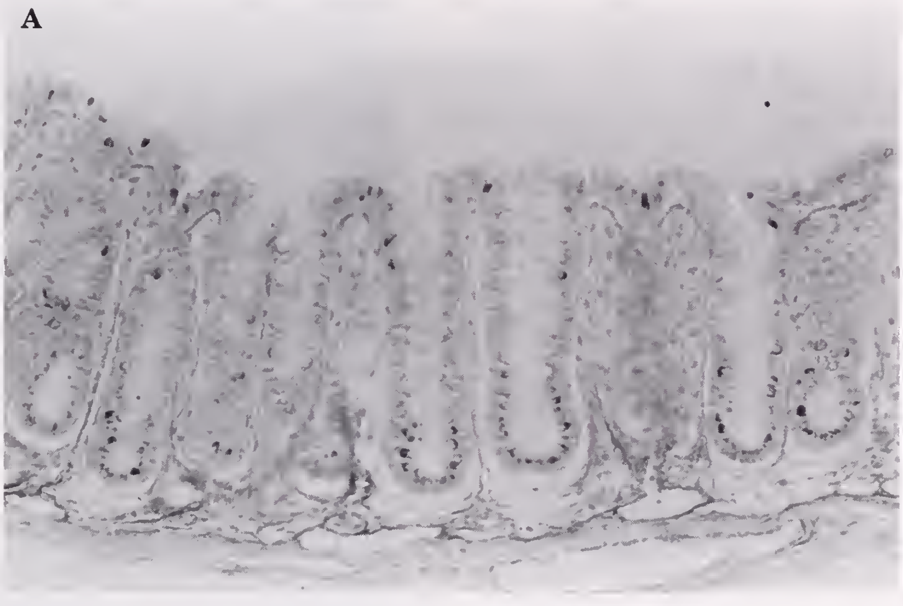
[d] $p < 0.02$ compared with control rats.

[e] Data designated in PC are significantly different from each other; $p < 0.05$.

Proportion of cells per crypt compartment binding ACA shown as (+) 1% to 25%; (++) 26% to 49%; (+++) 50% to 75%.

sity and prevalence in and near the crypt base but some positive cells were located toward the crypt surface (Fig. 1A). With starvation and refeeding, ACA staining intensity was altered markedly. In starved rats, staining intensity was significantly decreased throughout the crypt except in surface cells. After one day of refeeding, however, staining intensity was restored to near baseline levels. Although starvation did not alter the crypt compartment distribution of ACA positive cells after refeeding, a more uniform staining pattern was seen throughout the crypt.

In the PC, in control conditions, ACA binding intensity and prevalence were greater in the middle-third compartment as compared with the lower third and surface (Fig. 1B). Unlike the DC,



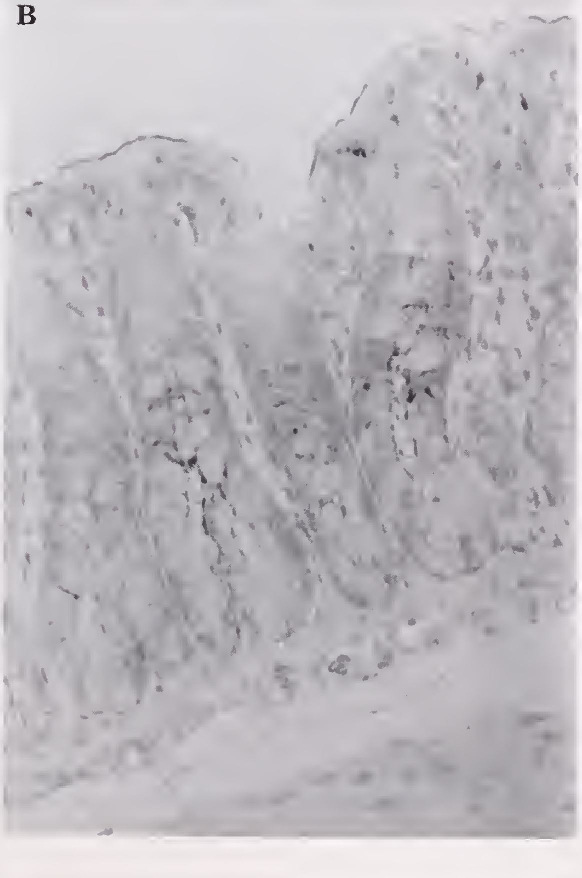


Fig. 1. Lectin histochemistry with ACA in control rats. (A) In DC, ACA binding occurs predominately in the lower third of the crypts in a supranuclear localization within the cells (magnification × 100); (B) In PC, ACA binding occurs mainly in the mid crypt region. Reactivity is in lateral and apical membranes as well as in the supranuclear region of some cells (magnification ×250).

however, starvation and refeeding in the PC did not substantially alter ACA binding intensity or prevalence in any of the crypt compartments.

Subcellular ACA Binding Patterns

ACA binding pattern within cells differed according to the region of the colon. In the DC, ACA binding was observed principally in the supranuclear cytoplasm of goblet cells with no binding to cellular membranes (Fig. 1A). Supranuclear binding appeared as discrete rounded structures which were usually filled completely in control conditions. With starvation, however, the supranuclear structures in most specimens exhibited a central core of lucency (Fig. 2A). This pattern reverted back to normal, filled structures after refeeding (Fig. 2B). The subcellular pattern for each of the respective nutritional states in stained cells showed minimal variation.

A different subcellular distribution of ACA binding was noted in the PC (Fig. 1B). In addition to maximal binding in the midcrypt region, ACA binding occurred rarely in the supranuclear cytoplasm. Instead, binding occurred as a thin streak at both apical and lateral aspects of goblet cell vacuoles with occasional distinct small pinpoint collections. This subcellular pattern of ACA binding in the PC did not change with starvation or refeeding.

Analysis of Proliferative Compartments

The identification of proliferative zones in the rat DC and PC are shown in Table 2. Data are only shown for lower-third and middle-third crypt compartments because proliferation did not occur above these zones.

In DC, ^{3}HdThd- and PCNA-stained cells were found predominately (58%–68%) in the lower third of the crypt with only 30% to 42% of proliferating cells extending into the middle third. Starvation resulted in a decrease in proliferating cells without a significant change in compartmental distribution. This was particularly striking with PCNA staining. Proliferative activity returned to control levels after one day of refeeding.

In the PC, the majority of replicating cells were found in the middle-third compartment. Refeeding resulted in a 52% increase in the ^{3}H labeling index compared with that found in starved rats, but as in the DC, there was no significant change in the crypt compartment distribution.

Discussion

Biologic differences exist between the proximal and distal colon that most likely reflect the

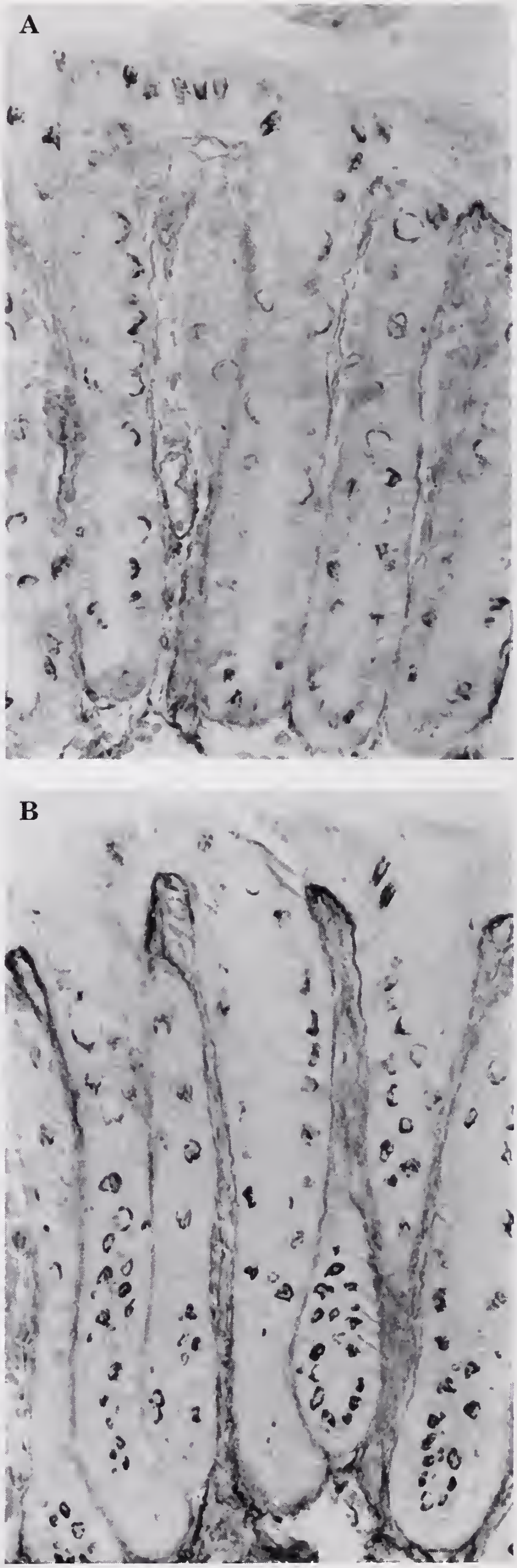


Fig. 2. Effect of starvation and refeeding on ACA binding in DC. (A) Starvation results in decreased ACA binding intensity and a central core of lucency in the supranuclear region (magnification ×400); (B) Refeeding results in an increase in intensity and refilling of central cores (magnification ×400).

TABLE 2
Effect of Starvation and Refeeding on Crypt Cell Proliferation Markers[a]

	Lower third crypt		Middle third crypt	
Proliferation markers	Distribution of labeling index (%)	Distribution of proliferating cells (%)	Distribution of labeling index (%)	Distribution of proliferating cells (%)
Distal Colon				
^{3}HdThd				
Control	38.9 (4.7)	68.6 (2.2)	20.8 (4.8)	30.9 (2.3)
Starved	31.3 (6.5)^{b}	63.4 (3.7)	18.0 (0.5)	36.6 (3.7)
Refed	49.2 (3.8)^{c}	60.4 (60.4)	32.3 (14.7)	39.6 (4.8)
PCNA				
Control	42.0 (7.5)^{d}	64.0 (2.8)	11.4 (2.3)^{f}	36.0 (2.8)
Starved	7.9 (3.9)^{d,e}	58.0 (6.4)	5.8 (1.2)^{f,g}	42.0 (6.4)
Refed	48.2 (7.5)^{f}	66.0 (1.0)	26.4 (4.6)^{g}	34.0 (1.0)
Proximal Colon				
^{3}HdThd				
Control	9.9 (1.1)	26.9 (1.6)^{c}	21.2 (2.7)	71.7 (2.2)
Starved	9.3 (2.8)^{b}	33.7 (3.3)^{c}	18.2 (2.1)	56.3 (2.9)
Refed	15.3 (1.8)^{b}	35.8 (3.4)	27.6 (5.4)	64.2 (3.4)
PCNA				
Control	2.2 (0.8)^{d}	10.9 (2.5)^{f}	16.2 (1.9)	89.1 (2.5)^{h}
Starved	0.4 (0.2)^{d}	3.2 (2.1)^{f}	12.8 (0.5)^{g}	98.5 (0.8)^{h}
Refed	6.8 (1.3)^{e}	21.4 (2.3)^{f}	24.2 (2.1)^{g}	77.8 (3.1)^{h}

[a] Mean labeling index ± SEM (parentheses) from a minimum of 20 crypts in 4 rats (3 rats for DC refed group). Distribution of proliferating cells determined from the percent of total crypt PCNA positive and ^{3}H labeled cells in each crypt compartment.
[b–h] Data designated by the same letter in either PC or DC are significantly different ($p < 0.05$); see Table 1 legend for explanation.

different embryologic origin of these two colonic regions. Differences between the PC and DC have been described with respect to morphology (9), proliferation (7, 10), fluid and ion absorption (11), and glycoconjugate expression (12). Cancers of the colon also manifest regional differences in features such as cell surface antigens (12), and molecular genetic alterations. For example, tumor suppressor gene deletions occur mainly in distal colonic cancers (13), whereas microsatellite instability occurs predominantly in cancers of the proximal colon (14). This suggests that the pathway(s) to malignancy and the influence of luminal factors might differ depending on the region of the colon.

Regional differences in glycoconjugate expression in normal colonic tissue from humans (15, 16) and rodents (17, 18) have been well described. These lectin binding studies not only highlight regional differences, but some lectins bind to cells in specific crypt compartments, suggesting that they are markers of cellular differentiation. An in-depth study of whether lectin binding might also be a marker of cellular proliferation has not been done.

The amaranthin lectin binds to the putative carcinoma-associated T-antigen and its sialylated derivatives (3). Studies in specimens of normal human colon have suggested that ACA binding, because of its lower crypt location and correlation with BrdU staining, may be a marker of cell proliferation (4). Additionally, ACA binds to most colon cancers. Other investigators, however, have questioned the concept that ACA binding is a reflection of cellular proliferation because binding in the human proximal colon was seen diffusely throughout the crypt (5) in their experiments.

Because ACA binding has been observed in the fetal rat colon (6) and the rat colon is a useful model for studying proliferation changes in response to dietary manipulation (7, 8), we examined ACA binding in a fasting-refeeding model. This was possibly the first study to look at regional expression of cellular glycoconjugates in the colon in differing nutritional conditions. Our results indicate that in the distal rat colon, the percent of cells binding ACA and the intensity of binding was greatest in the lower third of the crypts and decreased in other cells including those at the crypt surface. In the proximal colon, ACA binding was maximal in the midcrypt compartment. The greater reactivity of ACA in the lower crypt of the DC and in the midcrypt of PC was similar to the crypt distribution of proliferating cells in these two regions of the colon. Because ACA also may be expressed by cells at the crypt surface in both of these regions, however, suggests that ACA may not simply be a marker of proliferation but may also be associated with other factors including cellular differentiation.

The observation that ACA binding patterns in the DC were altered by starvation and refeeding suggests that lectin binding may be used as a probe of physiologic conditions affecting the colonic epithelium. The changes during starvation were mainly manifested as a decrease in staining intensity and a more hollow intracellular staining pattern, both of which resolved to normal after refeeding. Because the number of proliferating cells decreased with starvation, it is possible that the feeding-related changes in ACA binding are determined partly by changes in cellular proliferation. Differences in staining intensity may also represent quantitative changes in glycoconjugate production, because altered proliferation may uncouple glycosylation from peptide synthesis (19). Although ACA binding seemed to correlate with proliferation in control conditions in the PC, no appreciable change in response to starvation-refeeding occurred. It is possible that starvation may have slowed crypt cell migration (20) so that ACA-reactive glycoconjugates continued to accumulate in cell membranes. Whether differences in regional luminal factors or intracellular phenomena including apoptosis affect ACA-reactive glycoconjugate expression is not known.

The pattern of ACA binding in the DC, where binding was exclusively detected in the supranuclear area, suggests localization to the Golgi apparatus, the site of synthesis, or assembly of glycoconjugates. Although the intracellular binding was uniform in the fed state, a distinct area of central lucency was observed regularly after starvation. This suggests that there may be an alteration in the intracellular packaging of mucin vacuoles. Additionally, the *MUC*2 gene, a mucin gene cloned from human intestine, has several regions that are homologous with the D domain of prepro-von Willebrand factor, a domain that is considered important for the proper oligomerization, processing, and secretion of the mucin molecule (21). Whether ACA binds to carbohydrates on the *MUC*2 mucin is unknown.

In contrast to the DC, ACA binding in PC was not found in the supranuclear area, but occurred in, or close to, the cell membranes. Binding occurred principally to the apical membrane but was also seen in lateral membranes. There was little change in response to starvation and refeeding. Therefore, although glycoconjugate production or availability may be physiologically responsive to nutritional demands in the DC, this may not be the case in the PC, despite an increased prevalence and binding intensity in the midcrypt region.

Differences in intracellular binding between PC and DC suggest that there are distinctive regional biochemical forms or distinctive processing of the T antigen. Because the T antigen has been implicated as a putative tumor-associated antigen in the human colon, the differences in ACA binding patterns highlight regional differences and may reflect different neoplastic tendencies between the proximal and distal colon.

Acknowledgments

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Commencement Address

In Sickness and in Health

Christine K. Cassel, M.D., M.A.C.P.

Congratulations to our physicians graduating today. Shortly you will take an oath as part of that graduation. My message is drawn from another oath—a phrase from the traditional marriage vows—in sickness and in health.

I want to look first at health, a sound ideal for physicians, and ask us to apply the same scientific rigor to that goal as we do to caring for the sick; and I want to remind us of the centrality of caring for the sick in the mission of medicine.

Health has become the focus of medicine in the 1990s. We have been encouraged to turn from medicine, a "disease model," to health as a more holistic and positive goal. This is, in many respects, a positive move. We have realized that a goal of prevention leads us to consider ways to improve and maintain patients' health, rather than waiting for illness to occur. Modern understanding of the causes of illness, the ability to do sophisticated laboratory testing, more data (including genetic risk factors) available for counseling patients about health behaviors, and available treatment for preventing or postponing the onset of disease—all are great advantages.

Another way in which the language of "health" has overtaken the language of "medicine" is in our discussions about our own discipline. Because we understand the importance of the different health care disciplines and the essential contributions that they make, we become reluctant to single out medicine and the physician as having any unique role. So we call ourselves "health care providers" and in doing so perhaps diminish the importance of medicine and medical care in our thinking. We may not do so intentionally or explicitly, but nonetheless, the message is clear. How many times have you had the same experience, as I have had, of being at lectures or reading articles in which the term "medical care" has been changed to "health care," in which the term "physicians" has been changed to "health care provider?" Observers often accuse us of doing "sickness" care instead of "health care."

Part of the ascendancy of health in our language caused by the changes in health care delivery systems. The health maintenance organization, or HMO, as little as a decade ago was an interesting exception to the mainstream of fee-for-service practice. A few large HMOs were stable and successful, and they seemed to be rather unique, regionally limited entities that were unlikely to replace the varieties of medical practice existing around the country. Now, of course, we have "managed care," a loosely defined set of financing arrangements that systematically attempt to contain health care costs in a competitive market.

In support of managed care, the primacy of health and of prevention is often raised. Of course, in the original concept of the health maintenance organization, this was a fundamental and central value. Physicians being paid a monthly rate for the care of a defined population have a real incentive to maintain the health of that population and devise a system of services oriented toward that goal. Fee-for-service medicine never had those same incentives, and prevention depended on the physician's professionalism and scientific knowledge. To have effective health promotion and disease prevention in either setting, however, requires—more than any payment structure—a long-standing relationship with the patient. If that does not exist, little incentive exists for physicians to invest in the time and effort it takes to develop sets of preventive strategies for their patients. Even if physicians are interested, it is impossible to do so because they cannot get to know their own patients, the epidemiology of their risk factors and illness patterns, their attitudes and behaviors toward health promotion. Still, the ideology is a strong one and understandably popular. When we are healthier, we would much rather hear about programs designed to keep us healthy than hospitals designed to take care of us when we are sick.

Marketing experts tell us we should describe what we do as health care, not medical care. Look around at the billboards advertising HMOs and hospitals—they feature healthy-looking faces smiling out at us. The powerful subliminal message is that this health plan can keep you healthy, the power of association. It is like selling designer blue jeans by implying that, if you buy this brand, you will look like the model in the ad. Seems illogical, irrational, but advertising experts will tell you this technique works very well.

In my own specialty field, geriatrics and aging research, there is a counterpart to this phenomenon. We call it "successful aging." The tremendous, unprecedented, and unexpected increases in life expectancy that have occurred in this century have led to a remarkable phenomenon of numerous people surviving well into old age in quite good health, living independently and functioning well. Many of these people live with chronic illness, but they are not disabled by it and remain active. Researchers have developed various explanations for this successful aging phenomenon ranging from the genetic, to the behavioral, to the socioeconomic. All these factors play a role in determining who ages successfully and who does not. The message is clear that a considerable part of the risk of chronic illness in old age can be ameliorated by the personal attributes of people during their young-adult and middle-age years.

What's wrong with this picture? What's wrong with seeking health, valuing health, teaching healthy lifestyles, promoting healthy aging, selling health care instead of medical care?

One problem is that the evidence linking health so tightly with health care is not strong.

Is it the health care that makes us healthy—or not healthy? Hardly. Although it is useful to have a physician who educates you about healthy lifestyles and who maintains regular appropriate preventive screening practices, these factors play a small role in determining who is healthy and who is not. It is not all in the DNA we inherit either, although genetic influences are important and powerful. But more powerful than genes, and far more powerful than choice of health plan, are other determinants of health—specifically poverty and education. Extensive sociological research during the past 30 years has demonstrated repeatedly that a direct relationship exists between health and economic status. Recent studies in other developed countries show that there is an additional impact of poor health if there is a large gap between rich and poor—even if overall wealth is greater.

And these studies were done in countries that have universal health insurance. This is an important warning for the United States, where we have a growing income gap, a growing underclass, and the double whammy of a larger (and growing) number of people with no insurance. When we look at data on health and poverty, the skyrocketing stock market provides no comfort or reassurance. It won't help this problem, nor will all those billboards with smiling healthy faces.

Education, by itself, also has a forceful correlation with health status. Even within a constant level of income or wealth, people who are better educated have lower mortality rates and better health status. In developing countries, this is shown dramatically in the improved health status of communities when women achieve the ability to read—simply literacy.

Why do you suppose this is? In villages in West Africa, being able to read doesn't translate instantly into reading the instructions on a prescription or even a fitness or diet program. Most of these women will never encounter "health care" as we know it. Public health researchers speculate that it is a kind of empowerment that occurs, a partly psychological, partly spiritual, partly social and political phenomenon.

Whatever it is, this same effect is seen in the United States. At identical income levels, people with more education are likely to have better health.

These two factors, poverty and education, account for as much as three quarters of the difference in health status. This explains a major part of what the American College of Physicians has called the "urban health penalty" in a report on health problems of the inner cities, released a few weeks ago at a press conference with medical leaders and the Mayor of Philadelphia. Research shows that poor people living in our inner cities have worse health and die much younger than other Americans. Most of the illness and most of the deaths are not from violence, drugs, and crime. People suffer from heart disease, cancer, and stroke, the same as the rest of the population, but they are afflicted by these illnesses much earlier in life, and they die from them at a much younger age. Many do not have access to basic medical services, certainly not in the promise of "health care" beaming from those billboards. Many also suffer from the consequences of poverty and lack of education.

What can *we* do? What can this mean to physicians?

Please understand, I am soundly in favor of health, of health maintenance, of disease prevention and health promotion, and most certainly, of successful aging. We cannot alone correct the social problems of poverty and lack of education. It mattered less, however, when physicians took care of the sick and didn't bother so much with health. That has changed, and for the better. We should be concerned about health, but we should be honest and scientific about it. We should learn the real determinants of health, and we should partner with our colleagues in public health and join in advocacy for better education and better living standards. If we truly care about health, we must expand our horizons to include these social realities.

My other concern in speaking with you here today is that I believe that our enthusiasm for the rhetoric of health may have obscured the fundamental responsibility of the physician—that is, in caring for the sick. Advances in biomedical research have given us dramatically effective tools for diagnosis, amazing potential to cure previously hopeless diseases, and even when cure is not possible, we can often achieve unprecedented improvements in function and quality of life for people coping with chronic illness or disability. The precious and noble pursuit of research will continue to reveal important secrets and give us additional powers to heal.

And when disease cannot be cured and function cannot be regained, the physician is trained to focus his or her considerable skills on the art and science of comfort—the recently reinvigorated field of palliative care. If we forget our core mission of caring for the sick or diminish the centrality of it in our profession, the public we serve will lose a great deal. Because no matter how effective we are at educating people about healthy behaviors, no matter how effective our screening programs, no matter how vigilant and well organized our health maintenance organizations, and no matter how successfully some of us age, illness will always be among us—and senescence is part of the human genome—even if it hasn't yet been mapped precisely.

The risk of such great enthusiasm for the rhetoric of health is that sickness becomes an aberration, and the sick person becomes an outcast. One can see this phenomenon in the emphasis on personal responsibility in health. The sick person takes the blame for being sick. Ethicists call this "blaming the victim." Sometimes this even goes so far as people believing that a positive psychological outlook prevents cancer, and therefore anyone who suffers cancer simply was not thinking the right thoughts. Elderly people who suffer chronic disabling osteoarthritis, Parkinson's disease, organic depression, or colon or prostate cancer may feel that somehow they are failures in their own aging. From an organizational standpoint, the rhetoric of health may justify insurance practices that skim off only the healthy patients, eagerly accepting the same payment rate averaged across populations and leaving the sicker patients to be cared for by someone else, whoever will take them. And finally, at its most extreme, this focus leads to a disinterest in end-of-life care. The dying patient becomes a "treatment failure."

Our interest in improving the quality of care must extend to providing competent and compassionate care for patients who are dying. Ironic it is, that after decades of advances in scientific medicine, we still need to be reminded that human beings are mortal, that all of us must someday face death, and that medicine—yes, medicine, not health care—can provide symptomatic relief and human comfort to patients and their families who are facing this profound and often devastating event.

Two important observations need to be made about end-of-life care. One is that medicine knows a great deal about the relief of pain and other symptoms. The hospice movement has shown us how care can be organized to provide patient-centered attention wherever the patient may be, and yet we have fallen short by being unable to apply what is known to the vast majority of patients who die in this country.

The other side of the story is that a great deal more research needs to be done. If we look at the tremendous enterprise of research in this country, funded by the National Institutes of Health, as well as by pharmaceutical firms and the health care industry (not medical care) industry, we find very little research focused on symptomatic relief approaches to improving the care of dying patients—the broad and important area known as palliative care. Our blindness to this field indicates the denial we have exercised in our excitement with the important and compelling advances in potentially life-prolonging treatment. The recent attention to care of patients at the end of life that we have seen in this country, perhaps in response to the great nationwide debate over the ethics of the physician's role in active assistance in a patient's death, can be seen as important progress. Nonetheless, I worry that if we become too enthusiastic in embracing health as our goal, it becomes too easy to leave the sick to someone else.

I believe, with Frances Peabody, that the best care of the patient requires an ongoing personal relationship and continuity of care. That is most important when illness occurs. My definition of sickness is not limited to acute illness. Indeed, the sickness that is the greatest challenge to medicine is chronic illness, the chronic illness that comes with the success story of an aging population. We see this not only in aging, of course, but also in the prolonged life expectancy of individuals with formerly fatal diseases such as sickle cell disease, cystic fibrosis, and now, of course, AIDS. All of these have now become chronic illnesses with which patients must live throughout the rest of their lives. And physicians, not health care organizations, are best trained to help maintain function, relieve symptoms, avoid unnecessary medications and hospitalizations, and, most importantly, to *be there* when illness looms as a trusted expert in the scientific practice of medicine and as a professional capable of caring for fellow human beings who face the tremendous challenge to personal integrity that comes when the intactness of the body or mind—or worse, both—is shredded by disease. This is a noble goal, the goal toward which Peabody reminded us, that "the secret of patient care is caring for the patient."

To be a physician in this sense requires competence of the most remarkable kind. It requires an understanding of a very rapidly advancing scientific base. It requires a practical knowledge of how to recommend treatments with which one has some familiarity, and the courage and wisdom to know when to try out new, less familiar treatments, and how to include, the patients and their families in these decisions that so often become matters of judgment or personal preference. In addition to competence, a physician must have the humanistic skills of integrity, professionalism, and compassion. These are the foundation of the ancient oath you will pledge today.

This is also in some way a new kind of challenge to us because the success story of reduced costs of managed care, although it is wrapped up in the rhetoric of health and health care, is challenged most dramatically in a marketplace in which every incentive is to avoid the person who is sick.

We physicians can now, at the entryway to the twenty-first century, really help people who are sick. Of this we should be very proud. We have not, however, found a way to make health care a sustainable resource in our society and to make it available to all who need it.

So it works both ways—poor people are more likely to be sick and sick people are more likely to be uninsured. Although the cost to employers is declining, the out-of-pocket cost for patients is increasing, and the number of patients with no insurance at all also continues to rise. We cannot consider this a success story of health, and we certainly cannot consider it a success story in competent, compassionate, and professional medical care. We have important work before us. But I believe that when we (and you whom we honor today) care for the sick, we should recognize the heart and soul of who we are. We must also be stewards of the resources needed to provide this caring to all. This is both our greatest challenge and our highest calling

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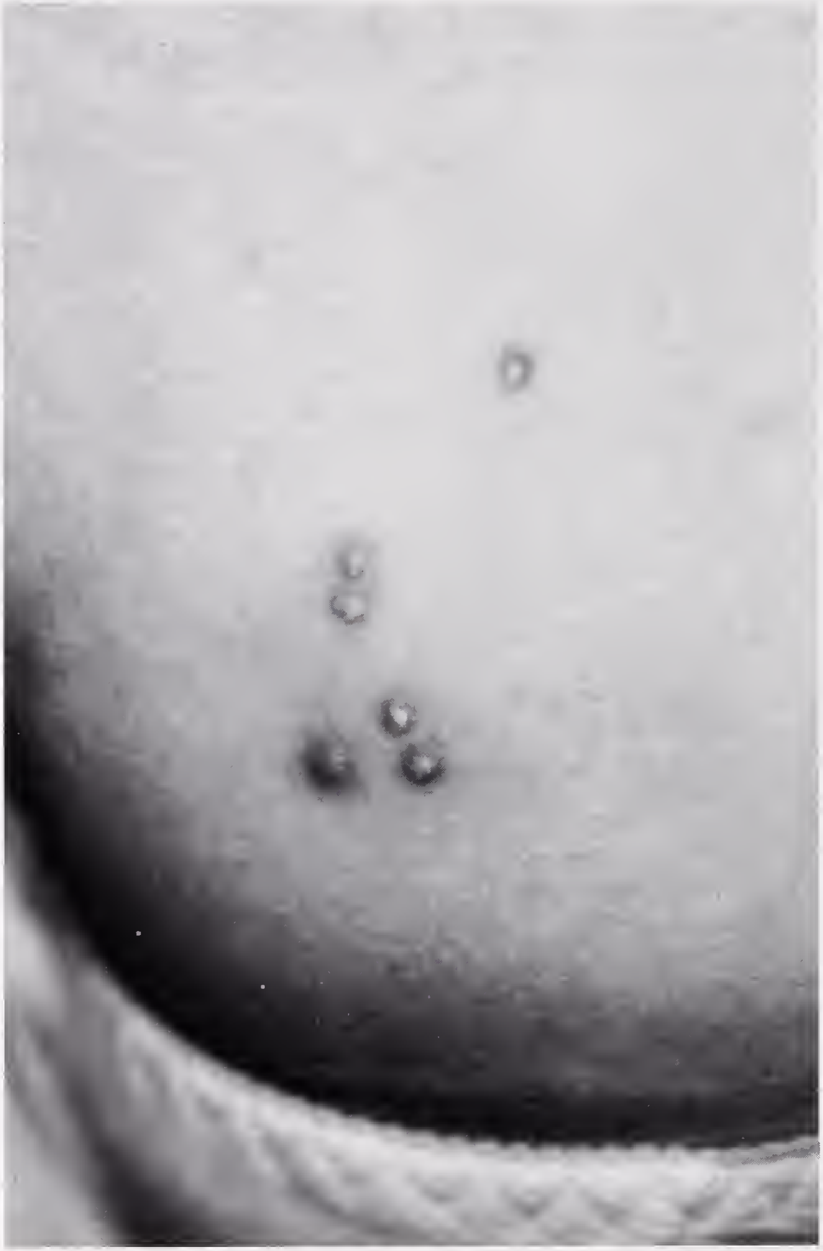
Delivered to graduating class of the Mount Sinai School of Medicine on May 16, 1997.

Dermatology Notes

Reactive Perforating Collagenosis



CHARACTERISTIC PAPULES with hyperkeratotic central plugs are shown on the back of a patient. The association between renal disease, diabetes, and reactive perforating collagenosis was first described at Mount Sinai. The histologic phenomenon of collagen fibrils penetrating the epidermis subsequently was verified in other patients reported as having as "Kyrle's disease." Many of those patients also proved to have diabetes and renal failure. The term "perforating disorder of renal disease" has been coined to include all of the perforating skin disorders associated with diabetes mellitus.—*Mark Lebwohl, M.D., and Stephen Kurtin, M.D.*

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VOLUME 65 NUMBER 3 MAY 1998

CONTENTS

THEME ISSUE

REPRODUCTIVE CHOICE

GENETIC COUNSELING AND ASSISTED REPRODUCTIVE TECHNOLOGY

Rosamond Rhodes, Daniel A. Moros, and Allyson Robichaud, guest editors

Introduction *Daniel A. Moros, Rosamond Rhodes, and Allyson Robichaud* . . . 159
Human Fertility and Differential Birth Rates in American Eugenics and Genetics: A Brief History *Kathy J. Cooke* 161
The Changing Moral Landscape of Human Reproduction: Two Moments in the History of In Vitro Fertilization *David A. Valone* 167
Reproductive Genetics and Today's Patient Options: Prenatal Diagnosis *Judith P. Willner* 173
Equivocal Notions of Accuracy and Genetic Screening of the General Population *Kelly C. Smith* 178
Ethical Issues Involving Multifetal Pregnancies *Richard L. Berkowitz* 185
Reproductive Choice and Public Policy: A Doctor's Perspective *Kurt Hirschhorn* 191
The End of Motherhood as We Have Known It *Patricia S. Mann* 195
Ideology and Technology: The Social Context of Procreative Technology *Barbara Katz Rothman* 201
Cases and Doubts *Randi E. Zinberg* 210
How Perfect? A Case of Prenatal Diagnosis *Barbra Paula Bluestone* 213
Panel Discussion 215

GRAND ROUNDS

Elmer's Glue, Elsie and You: Clinical Applications of Adhesion Molecules *Lynn M. Schnapp* 224

continued inside

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Veterans Affairs

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THE
MOUNT SINAI JOURNAL
OF MEDICINE

Volume 65
Number 3
May 1998

CONTENTS *continued*

A Personal Reflection on a Time Past *Mark M. Imberman* 232
Announcements of the 1997 Awards 234
Information for Authors 235
Required Form for Authors . . . . *inside back cover*

Introduction

DANIEL A. MOROS, M.D.
Associate Professor of Neurology
ROSAMOND RHODES, PH.D.
Associate Professor of Medical Education
ALLYSON ROBICHAUD, PH.D.
Medical Ethics Fellow

Issues in Medical Ethics: 1997

FEW ISSUES IN MEDICAL ETHICS have received as much public attention during the past 12 months as those related to human genetics and assisted reproduction. Remarkably, this intense focus is occurring while our society continues to struggle with the repercussions of two earlier science-based changes in human reproduction. One set of changes began in the second half of the nineteenth century and has lead to the stunning reduction in infant mortality rates and the drastic reduction in postpartum maternal injury and death. The other is the development of modern birth control techniques in this century. These dramatic advances are at least partly responsible for our increasing human population and the changing role of women throughout the world.

Ironically, in the Western world, expectations of likely survival for infants and mothers along with effective birth control have enabled women to postpone reproduction until the point where fertility becomes more problematic. Thus, control of reproduction has helped fuel the demand for an array of interventions to address infertility including artificial insemination, in vitro fertilization (IVF), manipulation of eggs and sperm to achieve fertilization, surrogate motherhood, and gamete donation. Each technique raises its own ethical issues.

Of course, as challenging and unsettling as reproductive issues have become, genetic research and the human genome project promises even more difficult issues. Human genetics will vastly expand our knowledge and capabilities within the next few years. In the near future, we expect to be able to screen fetal genetic makeup, not just for major genetic anomalies, but for a variety of less serious traits. Those abilities undoubtedly will leave us with many perplexing questions related to reproductive choice.

Abortion because of sex preference is already a reality in many parts of the world. Genetic screening of the embryo at an eight-cell stage is now available as an adjunct to IVF. Technology is leading us into new, uncharted moral terrain. Successfully navigating through the inevitable moral quandaries surely will require theoretical insight and compassionate understanding.

Yet, as science is leading us toward a new world, lawyers, legislators, and policy makers are attempting to guide the application of those rapidly expanding technologies which they do not fully understand. All this occurs in the context of media hype and commercial entertainment that capitalizes on the natural fear of change and the unknown and the fictional implications that can be linked to each scientific advance. In the United States and many other countries throughout the world, legislators and groups with political and social authority are busy drafting recommendations, regulations, and laws to prohibit human cloning and seriously limit the research that might make it a reality. Talk radio and talk television as well as the tabloids and even mainstream journalism eagerly capitalize on the sensational aspects and implications of each new technological breakthrough. All the media-manufactured hysteria impedes calm understanding and distorts rational public policy. Yet, the important ethical issues that emerge with the development of reproductive science need to be discussed in public forums.

The twelfth New York Regional Conference in Medical Ethics convened a multidisciplinary group of physicians, genetic counselors, philosophers, historians, and sociologists to consider the topic of "Reproductive Choice: Genetic Counseling and Assisted Reproductive Technology." As always, this conference, sponsored by the Mount Sinai School of Medicine and the Ph.D. Program in Philosophy at the Graduate School, City University of New York, sought to have health care practitioners and university-based academics challenge each other with their immediate practical problems and their broader philosophic perspectives. The historical papers by Kathy Cooke and David Valone provide a broad view of this country's history with reproductive and genetic technology. Papers by Judith Wilner, Richard Berkowitz, and Kurt Hirschhorn, all senior physicians, explore our current medical capabilities and related ethical concerns. Kelly Smith, a philosopher, explores how we deal with the uncertainty and ambiguity of the science. Barbra Bluestone, a physician, and Randi Zinberg, a genetics counselor, provide specific cases. Patricia Mann, a philosopher, and Barbara Katz Rothman, a sociologist, offer a more radical critique of our notions of family, parenthood, and society's responsibility to families and the disabled in light of the challenges of our evolving reproductive and genetics technology. As editors, we can only hope that the papers presented here will successfully communicate some of the constructive struggle that characterized this conference.

Human Fertility and Differential Birth Rates in American Eugenics and Genetics:

A Brief History

KATHY J. COOKE, PH.D.

Abstract

Eugenics is a broad term used to describe a variety of social and state-sponsored reform movements. Although we usually think of Nazi atrocities when we hear the word "eugenics," in this article I discuss the manifestations of hereditary reform worldwide. In particular, I consider the history of eugenics in America, focusing on concerns about the differences in birth rates between various racial, ethnic, and educational groups. In the early twentieth century, the social and cultural expectations that surrounded the growing knowledge in genetics implied an ethical imperative for physicians. Physicians were expected to use their knowledge about genetics to help them decide what sort of advice and assistance should be given to those who wanted knowledge about birth control, or help in resolving problems concerning sterility and infertility. Today, with growing knowledge about human genetics, physicians are subject to increasing pressure to make similar judgments. **Key Words:** Eugenics, history of medicine, genetics (history), hereditary diseases

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A Brief History

THE RELATIONSHIP BETWEEN EUGENICS, genetics, and assisted reproduction is not necessarily obvious. What is the connection? What relevance does the history of eugenics hold for medical ethics and assisted reproduction? They are related because the increasing ability in the late nineteenth and early twentieth centuries to control reproduction by way of birth control, and to increase it by way of treatment for infertility and sterility, brought with it concerns about who was having children, who should have children, and who should have the power to make decisions regarding reproduction. These issues all arose in the context of social concerns about national health and welfare, as well as concerns about the role of science in shaping culture. In this article, I will illustrate the efforts to control human fertility in American eugenics in the early twentieth century and attempt to draw some connections to the present day. In particular, I suggest that the social and cultural expectations which surrounded the development of American eugenics implied an ethical imperative for physicians in the early twentieth century.

Usually when one hears the word "eugenics," one thinks of the Nazi atrocities of the 1930s and 1940s. Hitler's efforts to annihilate Jews combined anti-Semitism with science, culminating in an attempt to change the genetic constitution of the population of Germany through the mass murder of millions of human beings. The history of eugenics in Germany vividly illustrates a combination of racism and science, and with terrible results. Here I will examine the American side of eugenics which predated the Nazi regime. I will consider its social roots in the context of the growing knowledge about genetics, and the interaction between the ideology of eugenics and reactions to

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Address correspondence to Kathy J. Cooke, Ph.D., Assistant Professor of History, Quinnipiac College, Box 77, 275 Mt. Carmel Ave., Hamden, CT 06518.

sterility and infertility, as well as birth control, in America from about 1900 to 1930. Through the historical experience in this country, we can see how eugenics and genetics relate to larger ethical questions in medicine specifically, and science generally.

Eugenics

The idea of breeding human beings to develop a superior population dates back at least to the Greeks. In its modern form, however, "eugenics" was founded by the efforts of the British aristocrat Francis Galton, cousin to Charles Darwin. Galton based his breeding schemes on the theories that he developed out of his research on "Hereditary Genius" in the 1860s and 1870s. In that work, Galton studied eminent families to see if their supposedly better qualities were passed along from parent to child. The research not only convinced him that genius was inherited, it also spurred him to develop a broader plan of action that would improve the biological traits of the British population. During the next few decades he, along with the mathematician Karl Pearson, popularized eugenics in England and America. By the 1890s, Galton was well known as an advocate of "better breeding," and he soon became known as the "father of eugenics" (1, 2, pp. 3–13).

Eugenics grew in popularity as scientific knowledge furthered understanding of the biological nature of heredity. August Weismann's theory of the immutability of hereditary material, which he called the "germ plasm," developed in the 1880s, and the rediscovery of Mendel's work in 1900 contributed to the development of eugenic theory by encouraging scientists to think of human attributes as physical material which specialized breeding could control and distribute almost at will. By the early twentieth century, Pearson and Galton had spread the gospel of eugenics throughout England (2, pp. 57–63). American agricultural researcher Charles Davenport built on their efforts in the United States and became the foremost American eugenicist. Davenport was especially confident about the possibility of breeding superior human beings with desired traits. He claimed that the "diversity of characteristics of people" along with scientific knowledge about inheritance meant that human characteristics could be combined to form "any desirable mosaic" (3).

Eugenic concerns also cropped up in other areas of the world, including France, Germany, and Brazil, with a variety of agendas (4, 5). In national contexts, worldwide eugenicists emphasized, to a greater or lesser degree, environmental influences on human development as well as strictly hereditarian influences. For instance, recent historical work on eugenics in Germany uncovered environmental reform efforts meant to improve the German population by changing behavior. German doctors, government, and media encouraged better diets and discouraged smoking (6). Thus even in the Nazi context, eugenics was not a strictly hereditarian movement, although the environmental efforts were imbued with racist ideology. In the United States as well, eugenics began as a movement that balanced concerns about heredity and environment, but by the 1920s and 1930s, strict hereditarianism seemed to dominate eugenic thought. It is this extreme hereditarian character of which most people think when they hear about eugenics. Still, American eugenics, like other national forms of eugenics, was multifaceted and defies a simple description as a "movement" based on biological notions of heredity.

Nevertheless, American eugenicists did share the racist, classist, and nativist ideology which characterized much of American society in the early twentieth century. White middle class Americans were concerned about the growing numbers of immigrants from Southern and Eastern European nations. They also worried about the rapid demographic changes in society, because the American people were becoming increasingly urban and industrialized. A variety of reform movements developed in America as a reaction to rapid social change as well as political corruption, all meant to bring about "progress." Thus, historians have come to call the period from about 1900 to 1915 the Progressive Era in American history. Progressive reform was extremely diverse, however (7, 8). Eugenic efforts began during the Progressive era and were one of many attempts to regain control over the rapid changes in American society. The nativism, racism, and class bias that permeated the early eugenics movement continued to characterize it, as it did American society in general, well into the thirties. Many of these biases, of course, persist to a greater or lesser extent today.

Although they disagreed about the relative influence of heredity and environment in human development, American eugenicists shared a concern about the future hereditary composition of the population of the United States. Frequently modeling their language on that used by farmers and agricultural scientists, they emphasized "breeding" among human beings. Eugenicists lumped human beings into groups with vague, ill-defined traits like "feeble-minded," "nomadic," "pauper," and "lover of the sea." Perceived as simple inherited characteristics, many American eugenicists believed that undesirable traits, such as feeblemindedness, could be bred out of the population in a relatively short time. Criminality and feeblemindedness were the targets of what are perhaps the best-known and most unnerving results of American eugenics: sterilization of individuals thought to be unfit and unworthy to reproduce and pass their traits along to their children. Efforts to sterilize the criminal were initiated by the aptly named Harry C. Sharp, a physician in an Indiana prison, in 1899, who performed vasectomies on his charges. By 1907, he had sterilized 465 men (2, p. 93). The Supreme Court considered sterilization of the "feebleminded" in the well-known case of *Buck v. Bell,* a test case for Virginia's 1924 eugenic sterilization statute. In the 1927 decision, the court upheld Virginia's law as it applied to the supposedly feebleminded Carrie Buck (2, pp. 110–111). In that case, Justice Oliver Wendell Holmes asserted, "Three generations of imbeciles are enough." Thirty states passed sterilization laws in the first half of the twentieth century, and about 60,000 legal sterilizations took place under this legislation. California performed more than half of the operations (9).

Differential Birth Rate

Although stories about sterilization are interesting and relevant, perhaps more appropriate to medical ethics and assisted reproduction were the concerns that American eugenicists frequently expressed in terms of the "differential birth rate." Eugenicists worried that poor American "stock" was reproducing much more quickly than individuals they considered "fit." Alfred Wiggam, one of the foremost popularizers of science in the 1920s, wrote about these issues at great length in his book *The New Decalogue of Science.* In that book, he compared the birthrates of schoolteachers and "bootblacks." Schoolteachers, if they had children, had an average of 2.2, whereas the number of children born to "bootblacks" approached 4 (10). Biologist William Castle made a similar point, explaining that he believed "the biologically poorest elements in the population may increase faster than any other," and furthermore, found this specter threatening to the future of the nation (11).

Eugenicists frequently emphasized the low birthrate among the highly educated. They noted that women with a college education married later and thus had fewer years to produce children. They argued that the numbers demonstrated a growing national problem. Princeton biologist Edwin Conklin explained that "at one of the leading women's colleges . . . the marriage and birthrate of the most brilliant students, who have been elected members of Phi Beta Kappa, is lowest of all." (12) James McKeen Catell was referenced widely for his claim "that a Harvard graduate has on the average three-fourths of a son, a Vassar graduate one-half of a daughter." (12) Conklin was not beyond making the differential birth rate seem apocalyptic; he claimed "the race that marries at 22 instead of 33 will possess the earth in two or three centuries." (12) This notion was intimidating to "old stock" Americans who saw immigrants marrying young, having numerous children, and thus in line to "possess the earth." (12)

According to eugenicists, differential birth rates clearly indicated that the so-called better classes needed to reproduce in higher numbers, whereas those of the so-called less desirable classes should have fewer children. As medical knowledge about fertility grew, specialists could play an increasingly important role in changing these birthrates. In the early twentieth century, physicians were acutely aware of eugenic concerns and social fears about the apparently changing composition of American society. Furthermore, many early advocates of birth control favored its use because they expected it to control population growth in desired ways, such as reducing the number of poor, and increasing the "better elements" of the population. Many opponents of birth control reversed the argument, however, claiming that only the wealthy and more highly educated would use birth control, thus further reducing the number of children they produced. Therefore, birth control was not a clear-cut eugenic issue. Whereas birth control could progress further in what was known as "negative eugenics," defined as efforts to reduce the number of undesirable births in the nation, it could also impede efforts to increase births in the desirable groups, known as "positive eugenics."

Concerns about birth control were closely related to questions about helping women and men who experienced infertility or sterility to have children. As historian Margaret Marsh and obstetrician-gynecologist Wanda Ronner pointed out in their recent book *The Empty Cradle,* "many of birth control's staunchest supporters were eugenicists who also advocated forced sterilization." Furthermore, they explain, "one might argue that any marriage of birth control and infertility treatment would have to be a way of trying to coax the middle class to have more children and the poor to have fewer." (13) Although Marsh and Ronner do not go on to make this argument, they do suggest the close relationship between infertility and eugenics in America, and indicate ethical dilemmas that must have affected physicians earlier in this century.

Although advice about birth control and infertility was not necessarily connected with the goals of eugenics, for many social reformers, biologists, and physicians, the two were intimately related. In fact, according to eugenic tenets, the physician had a moral responsibility to humankind to consider the eugenic implications of any assistance he might give. For instance, Paul Popenoe, former editor of the eugenic publication the *Journal of Heredity* and one of the country's most vocal eugenicists, related eugenic goals directly to the duties of the physician attempting to assist childless couples conceive. In 1926, he wrote *Problems of Human Reproduction.* In that book, he addressed fertility issues and asserted the responsibility doctors had to determine the eugenic suitability of parents who came to them for help. With regard to male sterility and the possibility of using artificial insemination to impregnate a woman with the sperm of a man who was not her husband, he wrote that the physician was "under a very heavy responsibility, from a eugenic point of view, to provide the best parentage possible; and equally to refuse motherhood to women who are not of good stock." The physician in this way also had the opportunity, Popenoe claimed (14), "to exercise an important influence on the eugenic welfare of the race."

Racism

The goals of this anticipated influence varied widely. Some reformers, especially the naturalist Madison Grant and paleontologist Henry Fairfield Osborn, were unabashedly racist and nativist (15, 16). They believed that eugenics should aim for a so-called purified Nordic race, in their minds strengthening the nation by returning to the "old stock" that had settled the nation in the seventeenth and eighteenth centuries. These men and their racist colleagues described new immigrants and people they found undesirable as a "swamp of human misery," "genuine human weeds," and "the scum of Europe" (17, 18). Thus, they found scientific justification for racist eugenics and discrimination.

Other eugenicists used growing knowledge about biology and heredity to attempt to value individuals based on their perceived genetic composition. They did not blame groups, they blamed a person's hereditary material and "protoplasm." According to Davenport "no race per se, whether Slovak, Ruthenian, Turk, or Chinese, is dangerous and none undesirable; but only those individuals whose somatic traits or germinal determiners are, from the standpoint of our social life, bad." In summary, Davenport (19) claimed that "immigrants are desirable who are of 'good blood'; undesirable who are of 'bad blood.' " Focusing on genetic traits rather than human groups, he explained that any race had traits that could contribute to the improvement of the American population. In particular, he believed that American Indians reflected inherited endurance and stoicism that could build up the American character through strategic breeding (20). Davenport's language (21), however, was as harsh as Grant and Osborn's when he claimed that American society had the right to "annihilate the hideous serpent of hopelessly vicious protoplasm," just as it had "the right to deprive the murderer of his life." Davenport was committed especially to maintaining the presence of so-called good protoplasm in the American population. Margaret Sanger (2, 22, 23) liked to claim that Davenport "used to lift his eyes reverently and, with his hands upraised as though in supplication, quiver emotionally as he breathed, 'Protoplasm. We want more protoplasm.' "

Although Sanger's story about Davenport was probably meant to ridicule the extreme nature of his eugenic convictions, it does demonstrate the centrality of increased reproduction among those considered "fit" by American eugenicists. Thus, Davenport was wary of birth control because of the reduction of births that it might cause in families that he considered to be the most sound eugenically. The availability of birth control, and questions about sterility, infertility, and relative birthrates in the American population preoccupied many American eugenicists. Physicians could help couples they considered "fit" to have children by using growing knowledge about fertility to solve infertility problems, and in this way further positive eugenic efforts. They also could encourage birth control among those they considered less fit, as well as withhold reproductive assistance for the unfit and sterile, furthering the goals of negative eugenics.

Thus Popenoe's 1926 claim that physicians had to consider the hereditary character of progeny reflected a larger social and cultural preoccupation with science and the growing knowledge in genetics, a preoccupation that seemed to imply an ethical imperative. As Popenoe asserted, physicians must use knowledge about genetics to consider group welfare in their efforts to distribute solutions to infertility and sterility. These conceptions of morality permeated the rhetoric of American eugenics. Many scientists, in fact, saw in eugenics the opportunity to serve society. Biologist William Kellicott, for instance, admonished his colleagues in biology to strive "to be useful in real human life." Further, he was happy to find that the biologist could feel "that he is at last in possession of facts of value to the student of human society . . . ." (24). This knowledge translated into a duty that students of society could apply "in order that evils may be lessened and benefits may be extended" (25). Kellicott, like many of his eugenically minded colleagues, believed that knowledge about the inheritance of human characteristics implied the duty to use that knowledge to achieve what they believed would be improvements for human society as a whole. Moral concerns also permeated the rhetoric meant to encourage voluntary cooperation with eugenic goals. Religious language and religious leaders, as well as contests to write eugenic sermons, were used to give eugenics "an ethical, altruistic, and religious turn" (26, p. 64).

The results of the eugenic ethic, reformers believed, would be a society freed from pain and suffering for the unfit whose birth would be prevented, as well as evolutionary progress for human beings. Thus eugenicists felt confident advocating "almost a racial religion for those with superior heredity to multiply more rapidly, and for those with inferior heredity to multiply less rapidly . . ." (26, p. 63). Moral responsibility did not lie exclusively with the physician, who could distribute information and assistance with regard to reproduction, but also with the individual who made decisions about whom to marry and whether or not to bear children.

These ethical and moral claims in the American eugenics movement, and similar claims in other national movements, bring to mind the obvious change that has come about in today's society. Today, rather than an ethical imperative toward eugenics, we experience demands to avoid eugenics. To avoid eugenics, one must know what eugenics is. It is easy, when one thinks of Nazi concentration camps and American sterilization efforts, to say what definitely must be avoided. However, when one considers the more complex issues that arise today—including the growing specter of fetal testing for traits not related to the health of the child, or the still remote but growing possibility of using in vitro fertilization techniques to adjust the genetic qualities of children—what is and what is not eugenics is becoming less and less clear.

An historian of modern genetics, Diane Paul, has raised this difficult question in a recent paper on the relationship between eugenics and human genetics, and has noted that today it is far too easy to say one opposes eugenics, thus obviously becoming a "good guy," without being specific about what eugenics is. Paul proposes alternatives, but does not provide a simple answer. Instead she suggests that we cannot easily dismiss a procedure or policy as eugenics, or off-handedly deny that something like the Human Genome Project could lead to a form of eugenics, without thinking about what eugenics might be in the context of growing knowledge in genetics. Eugenics cannot be a shorthand term that allows us to avoid thoughtful debate and consideration about issues related to human heredity and reproduction. With this in mind, I think we need to realize that the growing knowledge about genetics does now bring with it social pressures as well as personal dilemmas, conscious or unconscious, about how to use that knowledge, as it did in the early twentieth century. Those social pressures inevitably tend toward a sort of eugenics, or efforts to improve human welfare, be it individual or group, according to genetic knowledge or genetic preconceptions. Try as we like, we cannot ignore the implications which that changed knowledge has for human behavior. The possibility of fully understanding human genetics, or the option of cloning, suggests to many that human beings may be able to control their genetic destiny. Certainly increased knowledge of genetics at the turn of the century led many American reformers down that path. With changing knowledge about genetics, that challenge will only increase.

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The Changing Moral Landscape of Human Reproduction:

Two Moments in the History of In Vitro Fertilization

David A. Valone, Ph.D.

Abstract

Ethical attitudes toward in vitro fertilization (IVF) have varied historically. This paper discusses the changes in views about the morality of human IVF by exploring the response to a pair of critical moments in the history of this reproductive technology: the announcement of the first successful IVF experiment by John Rock and Miriam Menkin in 1944 and the first successful IVF live birth of Louise Brown in 1978. By exploring public and private reactions to these two events in the context of wider social and political developments, this paper demonstrates the degree to which our notions of the morality of medical experimentation and of reproductive technologies are socially constructed and historically contingent. **Key Words:** In vitro fertilization, ethics, medical (history), human experimentation, history of medicine

The First In Vitro Fertilizations

In the summer of 1944, two Boston researchers announced the first successful fertilization of human ova outside of the human body. John Rock and Miriam Menkin, associated with the Harvard Medical School in Boston and the Free Hospital for Women in Brookline, Massachusetts, had been toying for years with techniques for removing ripened ova from patients undergoing hysterectomies. By scheduling their procedures to take place just before the patient was to ovulate, Rock and Menkin had collected successfully many viable ova, which they could then maintain outside of the body in the donor's serum. Rock removed the ovaries, after which Menkin was responsible for isolating the ova. These ova were then exposed to sperm donated by willing medical interns. After nearly 6 years without success, Menkin noticed for the first time, in February 1944, that one of the eggs had been fertilized successfully. After repeating this successful procedure twice more, obtaining two- and three-cell stages of development, they published their results in the August 4th edition of *Science.* Two days later, a popular account of the discovery was carried on the Associated Press wire as a brief article; this news, however, was far from the stuff of major headlines (1–3).

Thirty-four years later, in contrast, the birth of Louise Brown in London was front-page news around the world. The story had broken some 2 weeks earlier, when Drs. Patrick Steptoe and Robert Edwards had held a press conference to notify the public that their decade-long attempt at in vitro fertilization (IVF) would produce a live birth. Well aware of the storm of publicity that their announcement would create, the doctors urged that "in the weeks ahead an atmosphere of total calm is vital to the well-being of the mother and her child-to-be." As Edwards and Steptoe surely anticipated, such pleas for tranquillity went totally unheeded. Even before the actual delivery of the baby on the July 26th, an active debate was taking shape between those who hailed the news as a great leap forward in the effort to

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Address correspondence to David A. Valone, Ph.D., Assistant to the Dean of Liberal Arts, Quinnipiac College, Box 77, 275 Mt. Carmel Ave., Hamden, CT 06518.

overcome infertility and those who saw it as heralding the arrival of Aldus Huxley's *Brave New World* (4).

These two events represent two of the most significant moments in the history of assisted reproductive technology. In this paper I will assess aspects of the moral response to these events and roughly gauge the climate of opinion at these two moments regarding the ethical implications and moral standing of these procedures. The purpose here is not to attempt to evaluate the ethics of these events in any absolute sense, but rather to remind us of the way in which moral attitudes are socially constructed and historically contingent. I also want to comment on the historical developments that occurred during the 30 years between these events which helped to shape the different responses that each received. In particular, I will discuss the implications of the debate over abortion that swept across the United States in the 1960s and 1970s.

Each of these events represents the culmination of countless years of research and the efforts of many people spread across at least two continents. In focusing on these two moments and their reception in the United States, I do not mean to ignore the communal nature of the research that shaped these events or the significance of the international response to them. Nonetheless, it is appropriate to consider these two events as vehicles through which we can judge the timber of public morality and its change through time. In undertaking this type of analysis, I do not wish to detract from more abstract philosophical discussion of reproductive technology. This topic is both appropriate and important, and other papers in this issue of the journal address it explicitly. I do want to try to emphasize the role that public perception of these medical techniques must play in their development and implementation, however, and to show that these notions can change radically during a relatively short time. In so doing, I hope to remind us again of the power of communal mores over the day-to-day application of medical technology, particularly in the area of reproduction, and to warn that such perceptions are quite changeable.

Public Reaction to the First In Vitro Fertilizations

First, I want to discuss reactions to the first successful IVF, announced in the summer of 1944, in more detail. Recall, now, something of the social and political climate of that era. The Second World War had been raging for about 5 years, and America had been involved for slightly more than 3 years. It was clear that the tide of the war was beginning to turn. Germany was on the retreat, and Allied forces had secured a footing on the European continent in both Italy and, more recently, in France. In the Pacific, the Japanese too had been turned back after their stunning early success, but the bloody island hop toward mainland Japan had just begun. At home, the industrial and technological forces of the United States had been combined with the willing participation in the war effort of millions of American women to produce a formidable war machine.

The announcement by John Rock and Miriam Menkin, then, of human fertilization outside of the human body was hardly the kind of news to tear America's attention from the war effort. It was the subject of a one-column story toward the back of the August 6, 1944 edition of *The New York Times.* The article itself did little more than to report the results under the prescient subheading "Test-Tube Babies." The report even took some luster off of the novelty of the announcement, noting that similar results had been obtained on one occasion 6 years before by a doctor in Philadelphia. The article made no effort to judge the scientific or medical significance of this feat, nor did it comment on any moral implications. However, the article did recount the story of the "near-riot among medical men" that had followed the announcement by Gregory Pincus in the early 1930s that he had succeeded in producing a "fatherless rabbit" through such a test-tube fertilization (5).

It is interesting to note the very different tone of this 1944 article from that of the report concerning Dr. Pincus and his "fatherless rabbit" 10 years earlier. In that article, also in *The New York Times,* under the headline "Rabbits Born in Glass," appeared the more ominous subtitle "Haldane-Huxley Fantasy Made Real by Harvard Biologists." This 1934 article explicitly recounted the biotechnological vision of Huxley's *Brave New World* and went so far as to quote an extensive passage of Huxley's book discussing the process of embryo budding and mass replication. The article also gave credit to John Haldane for popularizing the idea of what was then called "ectogenesis," the process of fertilizing eggs outside of the body (6). The reception of Pincus's work here was clearly rather hostile. Coming close on the heels of the Depression, however, Pincus's work took place in a volatile political and social climate. There had been some talk in those years about the role that science and technology had played in bringing on the Depression, and earlier, during the late 1920s, there even had been calls for a moratorium on scientific research to allow for an ethical assessment of the rapid pace of change and to allow social conditions to adjust to new technology.

It was perhaps not surprising, in contrast, that the announcement by Rock and Menkin in the 1940s fared somewhat better in the popular mind. Science and technology were seen as central to the war effort, and the race to stay ahead of the Nazis in the production of war-related technology had become something of a national obsession. This national mind-set laid the groundwork for a generally positive, although subdued, reception of Rock and Menkin's work. *Time* magazine opened its brief article on the discovery with an assessment of human nature: "Man will never be happy until he has proved that he is at least as smart as nature." The article also clearly demonstrated the gender politics of science in the 1940s, noting that Rock "and his assistant" Menkin were reporting their findings, even though the *Science* article listed them as coauthors. Perhaps not surprisingly, it was Rock who was inundated with queries from the press and other interested parties. Another revealing aspect of this article came in what might be best characterized as a comic aside, a statement that this discovery represented a "scientific affront to womanhood." The assumption of the 1940s clearly was that reproduction was the epitome of a woman's life (7). That such an assumption could be made in the 1940s is hardly surprising, but the lingering effects of this notion does raise the specter of the sexual politics of reproductive technology, an issue that will be addressed more explicitly by some of the other papers.

The potential for more steadfast support for Rock's work came from concerns that had been expressed during the war regarding the severe depopulation of Europe that the conflict would cause. In a 1943 conference in London, spokesmen for the Nazi-occupied areas of eastern Europe expressed concern that because of the mass extermination of males by the Germans in their homelands, it would take their nations more than 50 years to rebuild their populations to the prewar levels. In the United States that same year, the National Research Foundation for the Eugenic Alleviation of Sterility proposed artificial insemination of women as the best means to repopulate Europe while "improving racial quality" (8).

Rock's own desire to proceed with his work on human reproduction was removed far from these kinds of motivation. Instead, his interest in the problem was a product of his desire to help his infertile patients to achieve their dreams of having children. It also was connected directly with Pincus' earlier success. Rock and Pincus were good friends, and it had been Pincus' triumph with rabbits which had spurred Rock's work on humans. Rock had spent years working with women who had infertility problems. He saw in vitro fertilization as a technique that would hold out hope for the many women he treated with apparently insurmountable fertility problems. In the publicity that followed the announcement in *Science,* Rock strove to limit the hopes of those who envisioned the possibilities stemming from his and Menkin's work. He cautiously suggested that their work might someday provide hope for women with otherwise untreatable infertility. But he also vigorously denied that science would be able to raise such test tube embryos into viable infants (9). Despite his own caution on the subject, infertile women were quick to latch onto IVF as a possible solution to their problem; Rock was inundated by letters from women across the country pleading for information and help (3).

A Scientist's Personal Concerns

On a personal level, Rock was well aware of the delicate moral ground on which he was treading in his research. A devoted Roman Catholic, Rock worried about how his work in both IVF and his later work developing a birth control pill would be received by both the Church and the lay public. During the course of his and Menkin's experiments trying to achieve fertilization outside the body, Rock went so far as to discuss his procedure with both a Jesuit scientist and a Jesuit moral theologian. Although he could not win their unqualified endorsement, he was himself assured that "the only sin involved in all the work was committed mainly by him who masturbated to give me semen for the in vitro work, and by me in requesting the semen." Despite these seeming misgivings from Church authorities, Rock (10) resolutely maintained that "in this matter, regardless of what the priests say, my conscience is perfectly clear."

The letter that precipitated this admission from Rock was a heartfelt note from a former laboratory assistant, Katherine Moore. She had been receiving instruction from a priest while planning to join the Catholic Church. In the course of their discussions, she mistakenly had led the priest to believe that Rock had been removing fertilized eggs from the body of women, a slip that made her "dreadfully upset by the possibility I may have said something which might be distressing to you [Rock].” Moore was concerned especially that her error might have “unintentionally cast aspersions on the moral and legal integrity of a truly fine gentleman. By thoughtless misstatement I had made it seem that a mortal sin had been committed.” In the end, Moore (11) discontinued her instruction in the faith, but only after taking partial consolation in the belief that in aiding Rock, she had not presided over the beginning and end of a human life, but rather had only witnessed “the 3rd stage cellular reaction by division of human ova which had been activated outside of the body by laboratory procedure.”

Moore’s worry over committing a mortal sin was certainly an indication of her awareness of the ambiguities concerning the creation and destruction of life which surrounded Rock’s work in IVF. In this vein, her clinical description of the IVF experiments apparently was psychologically satisfying to her and helped her to resolve her guilt. As for Rock, he had apparently come to his own private moral closure with his work some time earlier, at least regarding the experimental procedures. This might have been easier for him as a result of his having had a regular medical practice, in which he was accustomed to finding and removing fertilized embryos from the uteruses of women upon whom he was performing medically necessary hysterectomies. Whenever possible, these embryos were carefully removed and preserved for preparation into slides that could illustrate very early stages of embryo development in humans. Rock (12) liked to tell a story that to some degree explained his accommodation to this medical reality. It involved his going to confession at an early age and being told by the priest: “John—don’t be so scrupulous.” The young John Rock needed to run home and look up the priest’s sophisticated word, but the notion clearly provided him with some comfort traversing the rocky moral terrain over which he was later to walk. Publicly, however, these moral complexities of IVF were not discussed much, and they would remain largely dormant as the scientific investigation of conception and early human development continued during the next two decades.

Reaction to Louise Brown’s Birth

In contrast, the furor over the birth of Louise Brown some three decades later took place against a very different public mood and the outcry over the event clearly demonstrated the difference between research which was essentially private and the public uses to which it could be put. During the 30 years between the first successful IVF and the first successful IVF live birth, vast changes had occurred in the public perception of medicine, in the social context in which medicine was practiced, and in Western society itself. The most relevant change, of course, was the vast revolution which had gone on during the decade of the 1960s and 1970s in sexual mores and in the struggle for women’s liberation. These had transformed completely the way in which sex and reproduction were viewed on a social level, but only by raising it as an issue of major controversy. On the heels of this battle came the fight for the sanctioning of abortion as a legitimate, and legal, medical procedure. A resulting wave of legal reform swept over the Western world, erasing repressive abortion prohibitions and opening the door to widespread abortions (13). These battles, and the bitter debates that surrounded them, clarified the critical role of reproduction not only in the individual lives of women, but as one of the fundamental threads in the social fabric.

The announcement of the impending birth of the first test-tube baby, therefore, entered a social climate already sensitized to the controversy engendered by reproductive issues. The atmosphere was clouded further in the United States by a coincidental legal battle that was reaching a crescendo that same summer. This involved a suit by Doris Del Zio, a woman who in 1973 had been trying to have a test-tube baby with the aid of Dr. Landrum B. Shettles. A vial containing fluid aspirated from Del Zio’s ovaries mixed with her husband’s sperm had been seized by Raymond L. Vande Wiele, Columbia-Presbyterian’s chief of gynecology. Vande Wiele had interrupted the procedure on ethical grounds, declaring the work to be an unsanctioned experiment on a human subject. The Del Zios sued on the basis of their claim that this mixture could have contained a viable zygote (14, 15).

Ethical Concerns of In Vitro Fertilization

The trial raised the issue of the connection between human experimentation and IVF. Possible abuses of human experimentation had been the subject of increasing public debate during the previous decade. Central to fueling the fire of these debates had been the Senate Subcommittee on Health’s hearings on Human Experimentation in 1973, which focused attention on the Tuskegee Syphilis Study and the clinical trials of Depo-

Provera. Shortly afterward, a moratorium was imposed on human IVF research in the United States, resulting from a decision by the recently formed National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research. Early in 1978, the National Ethics Advisory Board was formed, providing a means of monitoring and clearing human medical experiments in the United States. The Board took up the issue of human IVF as one of its first cases, largely because the publicity surrounding the Del Zio's case and the birth of Louise Brown. The Board also began a series of public meetings around the country to discuss the ethical and legal implications of IVF work (16).

The Del Zio case and the public furor over the birth of Louise Brown made even murkier the cloudy waters surrounding the ethics of IVF as a laboratory experiment and as a clinical procedure. The most fundamental objection came on the same issue that had troubled John Rock and his assistant 30 years before: the destruction of fertilized human ova. In 1974, Robert Edwards, then at work on achieving the first live IVF birth, had sought to answer these issues preemptively in an article in the *Quarterly Review of Biology* (17). There, he argued that it was incorrect to try to establish a moment, like conception, when life begins. Choosing such an arbitrary point in the continuum of generational reproduction was a distortion of the "continuous developmental sequence." Limiting IVF work on this basis, in his view, was unjustified biologically and morally. This argument clearly carried some weight, especially given the now legal right of women to abort much more developed fetuses. Other equally troubling questions also were being raised, however, which were more difficult to answer. There was a great concern about the potential for birth defects arising from the processes, either born of the physical manipulation of the embryo or from possible joining of an egg and sperm that had not undergone the body's own selective screening process. The issue of multiple fertilizations and the destruction of viable embryos was also a center of controversy, as was the eugenic specter of potential genetic screening and selected implantation of chosen embryos. Edwards himself did little to dispel this last concern, noting in his *Quarterly Review of Biology* article (17) that, although the potential of human cloning was remote, "any method of potential value in raising human standards should be considered, and cloning might contribute toward this end by providing pools of talent."

Such eugenic rhetoric, needless to say, did nothing to dampen fears of the pessimists. Paul Ramsey was one of these more apocalyptic critics. He focused his concern on the potential for grave injury to the child—in the form either of a physical defect or a more subtle but no less serious psychological harm caused by the realization of being a medical marvel—and argued that it should present an insurmountable barrier to further IVF application. He even somewhat jokingly hoped for a monstrous IVF birth to serve as a model of the great dangers the procedure posed to the unborn and unconsenting children who were to be produced (18). Such objections may now seem alarmist in an era when IVF has become widely practiced. In the nearly two decades since the birth of Louise Brown, the public and medical perception of the procedure has changed from one of a risky experiment to a widely accepted option giving hope to infertile couples. In many ways, then, the vision of John Rock and his optimistic hopes for infertile couples has been fulfilled. The path that we have trodden, however, has been one that was full of serious ethical objections, many of which we now dismiss somewhat casually. But in an era when genetic manipulation has become a reality, the questions surrounding IVF need to be assessed once again. Especially with the recent announcements of successful cloning of higher mammals, the specter of Huxley's *Brave New World* seems to have risen in earnest. In that context, we would be well served by the memory that just as our medical abilities change with the times, so too do our moral attitudes. We would do well to consider which, our science or our ethics, should take the lead. And although we might like to view the changes in our moral attitudes during the past half century as a move toward a more humanistic ethics, further developments in IVF may begin to call into question our very understanding of what it means to be human.

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Reproductive Genetics and Today's Patient Options:

Prenatal Diagnosis

JUDITH P. WILLNER, M.D.

Abstract

Rapid and safe prenatal diagnosis has become the standard of care in high-risk pregnancy. The safety and reliability of prenatal diagnosis by mid-trimester amniocentesis and first-trimester chorionic villus sampling (CVS) are reviewed, and accepted medical indications for referral are defined for both procedures. Techniques for evaluating the fetus for abnormality including amniocentesis, CVS, ultrasound, percutaneous umbilical blood sampling, fetal biopsy, amniotic fluid α-fetoprotein analysis, and maternal serum screening are described. The need for appropriate prenatal genetic counseling before any diagnostic modality is emphasized. **Key Words:** Genetic testing, genetic screening techniques, prenatal diagnosis, fetal diagnosis, amniocentesis, chorionic villus sampling, fetal blood sampling, α-fetoprotein screening, genetic counseling

IN 1955, SERR, SACHS, AND DANON (1) described the feasibility of antenatal sex determination by the analysis of sex chromatin in amniotic cells. Ten years later, Steele and Breg (2) published their successful technique for the culture and karyotyping of fetal cells obtained through amniocentesis. In the 10 years between these two reports, significant advances had been made in the safety of mid-trimester amniocentesis. Technological improvements in the field of in vitro tissue culture permitted Steele to speculate that the confluence of these new techniques would allow families to decrease their risk of having a child with a genetic disease (2). Indeed, within a year (1967), Jacobson and Barter (3) described successful prenatal chromosome diagnosis in three pregnancies at high risk for chromosomal abnormalities. In discussions after the presentation of their paper, scientists were clearly aware of theoretical, legal, and moral repercussions which would need to be faced as prenatal diagnosis became part of clinical practice. The need to define carefully through statistical study, the "high-risk" pregnancy, in which these techniques were indicated most clearly, also was realized. In the decades that followed, careful, multicenter studies have determined that the common techniques of prenatal diagnosis, amniocentesis and chorionic villus sampling, are acceptably safe and that the results obtained are highly reliable (4–12). The number of disorders that are diagnosable prenatally is increasing continually (13, 14). In addition, experience in the field has exposed us to hazards and pitfalls, both technical and psychosocial, and revealed the need for ethical and legal consideration of the issues attendant to prenatal diagnosis.

Common Indications for Prenatal Diagnosis

The largest group of women referred for prenatal diagnosis consistently has been those at risk for cytogenetic abnormalities because of advanced age. The chromosomal aneuploidies recognized as occurring with increased frequency in the pregnancies of older women are the trisomies, specifically trisomy 21, trisomy 13, trisomy 18, 47,XXY (Klinefelter syndrome), and 47,XXX. By

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Address correspondence to Judith Willner, M.D., Department of Human Genetics, Box 1497, Mount Sinai School of Medicine, One East 100th Street, New York, NY 10029.

far, the most frequent disorder is trisomy 21 (Down syndrome) which has accounted for just more than 60% of all chromosome aneuploidies detected in the older age group (35 to >40 years). An analysis by Hook (15) of pooled data from four large-scale studies on the rate of Down syndrome in live births reveals that the rate rises sharply from 1 to 2 per 1000 in the 30- to 34-year age group to 3 to 4 per 1000 for women aged 35 to 39 years, and to 10 to 15 per 1000 for women aged 40 to 44 years (15). The risk is approximately 5% for women older than 45 years. The same data have shown that the rate of Down syndrome diagnosed at mid-trimester amniocentesis in women of advanced age is approximately twice that in live births (15). The data also indicate that the same is true for fetuses with trisomy 13 and trisomy 18 (15). (The best explanation for this discrepancy is that chromosomally abnormal fetuses are at high risk of aborting in the latter part of gestation.) These data are relevant to the counseling of couples considering amniocentesis, providing them with information about the risks connected with having conceived a chromosomally abnormal fetus.

The next largest group of women referred for amniocentesis are those who have previously borne a child with a chromosomal anomaly. The risk of detection of a chromosome problem has been identified for those women who previously have conceived a child with Down syndrome and is approximately 1% for women younger than 35 years.

A third group at high risk is couples in whom one parent is a carrier of a balanced translocation. The risk of conception of a fetus that is partially trisomic and monosomic for one or both of the chromosomes involved in the translocation is increased. The actual risk of an abnormal conception depends on the individual translocation and the ratio of the meiotic segregation product, and also, on the sex of the parent who carries the translocation. (Generally, the risk is increased when the mother is the translocation carrier.)

The presence of normal/trisomy mosaicism in a parent is usually ascertained through the birth of a trisomic child or through the screening of couples with a history of fetal wastage. The risk to these couples of a recurrence of a trisomic fetus is considerably higher than that from chromosomally normal parents.

Prenatal cytogenetic diagnosis is also of value to couples in whom the mother is a known or possible carrier of a serious X-linked recessive disorder. If the fetus is male and therefore at 50% risk for inheriting the mutant gene, further biochemical or molecular testing may be indicated. Prenatal cytogenetic diagnosis is often performed if there is a history of a previous child with multiple congenital anomalies when the diagnosis on that child was not known.

Prenatal diagnosis is offered to couples at risk for children with Mendelian disorders (autosomal dominant, autosomal recessive, X-linked) when it is known that the diagnosis can be made accurately on biochemical analysis of amniotic fluid, cultured chorionic villi, or amniotic cells, molecular studies on uncultured or cultured fetal cells, high resolution ultrasound examination of the fetus or a combination of these methods.

Prenatal diagnosis of multifactorial disorders (e.g., neural tube defects and congenital heart disease) may be performed through the assay of biochemical markers in amniotic fluid (α-fetoprotein) and high-resolution ultrasound or special ultrasound procedures (fetal echocardiogram).

Fetal Diagnosis: Techniques For the Acquisition of Fetal Tissue

Amniocentesis

Collaborative studies in the United States, Canada, and Europe have all testified to the relative safety of mid-trimester amniocentesis and the reliability and accuracy of the cytogenetic studies on cultured amniotic cells (4–9). The risk of the procedure determined by the United States collaborative study is approximately 0.5% (8). The studies also indicate that the least morbidity occurs when the procedure is performed between the 15th and 20th weeks from the last menstrual period. The procedure of amniocentesis always is performed under ultrasound guidance to avoid fetal or placental injury. Approximately 10 to 30 mL of amniotic fluid is removed. The risk from the procedure increases if more than two punctures to the amniotic sac are required to obtain adequate fluid. The fluid can be used for amniocyte culture, biochemical or molecular analysis, or microbial studies. Karyotypes on cultured amniocytes generally are available 7 to 10 days after the procedure. The time required for results on biochemical and molecular testing depends on the volume of tissue required (tissue culture time) and the type of molecular testing involved (direct mutation analysis by polymerase chain reaction [PCR] vs. Southern blot analysis). For some diagnoses, preliminary results may be available within hours of the procedure (e.g., assay of hexose aminidase A in amniotic fluid for Tay-Sachs or sex determination on uncultured cells by PCR for Y-chromatin in sex-linked disorders).

Chorionic Villus Sampling

Chorionic villus sampling is advantageous because it can be performed at an earlier point in gestation (9 to 12 weeks from the last menstrual period). Because the villus cells are, by their nature, rapidly dividing cells, a direct preparation (uncultured or short-term culture) often can be examined for rapid cytogenetic diagnosis. Long-term culture, time to diagnosis for biochemical or molecular testing, is the same as for amniocentesis. However, diagnosis is usually possible in the first trimester. The patient must be counseled that this procedure carries a greater risk than amniocentesis of 1.5% as compared with 0.5% for amniocentesis (10–12) and that a dating ultrasound for gestational age and fetal viability must be performed before the procedure. In addition, vaginal and cervical cultures for pathogens must be done before the procedure to avoid contaminating the uterine cavity. The procedure is performed under ultrasound guidance. The catheter with stylus is passed through the cervix and up against the fetal placenta. The stylus is then removed and villi are aspirated. If the placenta is posterior and inaccessible via the transcervical approach, a transabdominal approach is taken. This technique is similar to amniocentesis. However, in this case, the tissue biopsied is the fetal placenta or chorionic villi (12). After reports from two centers reporting an increased risk for fetal limb malformations after CVS (16, 17), three large-scale studies were undertaken to document this risk and to establish safety. No increased risk for limb defects was found when the procedure was performed at 9 to 10 weeks, when the catheter used was small, and when the operator was experienced (18).

Percutaneous Fetal Blood Sampling

Percutaneous fetal blood sampling (PUBS) is performed late in the second trimester for a limited number of indications: to document fetal chromosomal mosaicism, for microbial analysis of fetal blood, for fetal hematologic status (hematocrit, platelets), for immunologic status (immunoglobulins, white blood cells), or for metabolic status (pH, electrolytes, liver enzymes, serum proteins). The risk resulting from the procedure in the second trimester is approximately 1% (19).

Fetal Tissue Biopsy

Fetal tissue biopsy with fetoscopy and ultrasound guidance may be performed for the diagnosis of potentially lethal dermatologic disorders (bullous diseases), and muscular dystrophy and metabolic disorders not diagnosable on cultured cells. The risk resulting from this rarely performed procedure is at least 5% (20).

Maternal Serum α-Fetoprotein Screening (MSAFP) and Amniotic Fluid α-Fetoprotein Testing (AFAFP)

α-Fetoprotein (AFP) first was identified in human fetal blood in 1956 (21). It is the major circulating protein of the early human fetus and reaches peak concentrations of 2 to 3 g/L at 12 to 14 weeks of intrauterine life and thereafter decreases as pregnancy advances. The presence of AFP in the amniotic fluid of normal pregnancies was reported in 1966 (22). Most is derived from fetal urine. In 1972, the markedly elevated levels present in pregnancies with open neural tube defects (NTD) (anencephaly and meningomyelocele) were described by Brock and Sutcliffe (23). The mechanism by which it becomes present is presumed to be leakage from exposed fetal capillaries. The potential for early diagnosis for fetal abnormalities was recognized quickly (24). Standards for normal and abnormal levels of amniotic AFAFP at different stages of pregnancy were established. The maximum concentration in amniotic fluid occurs at 14 to 15 weeks (50 mg/L) and decreases to less than 10 mg/L after 22 weeks of pregnancy.

The development of a radioimmunoassay for AFP permitted the detection of AFP in maternal blood. This provided an effective means for screening all pregnancies for NTD. Other fetal anomalies have been found to be associated with elevated AFP. These include esophageal atresia, omphalocele, gastroschisis, and Finnish nephrosis. The differential diagnosis of elevated AFP also includes multiple pregnancies, fetal demise, and unaffected cases in which complications appear later in pregnancy. MSAFP screening will detect approximately 80% of open NTD and 95% of anencephaly. Confirmation of the screening test is obtained with high-resolution ultrasound for anatomic defects. Amniocentesis for confirmation of elevated MSAFP may be recommended if the ultrasound does not detect an anatomic defect. Acetylcholinesterase, an enzyme in the central nervous system which also becomes elevated in amniotic fluid with open NTDs, also is measured at the time of amniocentesis. Its presence helps to distinguish an open NTD from other fetal abnormalities (25).

More recently, the usefulness of an abnormally low MSAFP for screening for fetal abnormality was recognized. Abnormally low MSAFP levels have been found to be associated with fetal trisomy, particularly with trisomy 21. MSAFP screening for women older than 35 years currently is offered as a means of screening for Down syndrome in younger women who would not be seeking amniocentesis or CVS. Other chemical markers have been evaluated in an attempt to improve detection rate. At this time, human chorionic gonadotrophin measured simultaneously with AFP in maternal serum results in a detection rate of approximately 60% for trisomy 21. There is a 5% false-positive rate. The rate for detection of trisomy 18 is also approximately 60% with a 0.4% false-positive rate. When a high risk of fetal abnormality based on maternal serum markers is detected, several parameters that affect the level are considered in the establishment of an accurate calculation: maternal weight, maternal diabetes, and pregnancy dating. Amniocentesis for accurate cytogenetic diagnosis is offered to those women whose maternal screening markers have increased their risk for chromosomal abnormalities above their age-related risk (26).

The American Societies of Human Genetics and The American College of Obstetrics and Gynecology currently recommend that genetic diagnosis be offered and informed consent obtained before performing any form of prenatal diagnosis, including maternal serum studies. The counseling session should include obtaining a three-generation family history. An effort should be made to secure accurate diagnoses for disorders that are potentially genetic, because risks of recurrence cannot be determined without accurate diagnoses. Recurrence risks should be explained in language that is clear to the layperson. The accuracy and risks of the prenatal procedure under discussion must be explained to the patient and, in addition to prenatal diagnosis, other reproductive options (e.g., adoption, donor sperm or ova, etc.) should be discussed. It should be made clear that the decision to pursue prenatal diagnosis does not necessarily entail a decision to terminate the pregnancy if the fetus is found to be affected. Counseling should be offered in a nondirective, nonjudgmental manner. The patient should feel secure that the counselor will provide support regardless of the reproductive decisions made by the patient.

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Equivocal Notions of Accuracy and Genetic Screening of the General Population

Kelly C. Smith, Ph.D.

Abstract

The explosive growth in genetic technology quickly will make possible an unprecedented number of tests for genetically based conditions. A necessary condition for the use of such tests without risk of harm to the patient is their accuracy. However, most discussions of test accuracy in the literature have equivocated between two importantly different meanings of the word. In particular, it must be remembered that a high analytic accuracy does not imply a high diagnostic accuracy. Questions about the diagnostic accuracy of genetic tests presently loom large because of our limited knowledge of the complex etiology of disease and the distribution within the general population of the causal factors involved. Our current inability to supply patients with accurate diagnosis based on genetic information, however, is less problematic when examined in the context of new reproductive technologies such as in vitro fertilization and intraplasmic sperm injection. **Key Words:** Genetic testing, genetic screening, genetic determinism, reproductive technology, abortion, cystic fibrosis, BRCA1

> "For every complex problem, there is a simple, easy to understand, incorrect answer."
>
> —Albert Szent-Györgyi

Introduction

The growth in genetic testing technology is explosive. The Human Genome Project, which aims to sequence all 100,000 human genes, is currently ahead of schedule and should be completed by the year 2001—some even predict that we will have the sequence for all genes in all living organisms by 2015 if sequencing efforts continue at the present rate (1). Within the next 10 years, as many as 400 new genetic screens are likely to become available, aided by development of integrated circuit chips capable of performing thousands of hybridization probes on a single minute sample of DNA. Already, more than 4 million blood samples from newborn heel sticks are screened each year for various genetic disorders (2). Genetic screening thus is likely to be viewed increasingly as a common and accepted aspect of modern medicine, based largely on the perception that offering patients screening is itself a harmless enterprise and may even be required as part of the standard of care (3–5). However, offering genetic screening is often only harmless to the extent that it provides a basis for making informed decisions about one's condition. For example, a bilateral radical mastectomy may (or may not) be a justified procedure if one is at some uncertain risk for breast cancer.

Information and Accuracy of Generalized Genetic Screening

I do not mean to suggest that genetic screening is inherently evil, much less that it is avoidable. Also, it is beyond the scope of the present work to address all the various social and ethical issues that the widespread use of genetic screening in the general population will surely raise:

Address correspondence to Kelly C. Smith, Department of Philosophy and Religion, Clemson University, 101 Hardin Hall, Box 341508, Clemson, SC 29634-1508.

access to insurance, privacy, social stigmatization, eugenics, etc. However, I do want to draw attention to a problem with genetic screening which has not received as much attention in the literature as it deserves (6, 7).

The problem is that discussions of the accuracy of genetic testing often equivocate between two distinct meanings of "accuracy." With our current state of knowledge, a high degree of analytic accuracy in a genetic test does not imply a similarly high degree of diagnostic accuracy. By "analytic accuracy" I mean the accuracy with which a test identifies the presence of the gene it is designed to locate—avoiding both positive results when the gene is absent and negative results when the gene is present. By "diagnostic accuracy" I mean the accuracy with which the test can be used to make predictions about a patient's prognosis. For the sake of argument, let's assume that we are only discussing tests that are reproducible and have a 100% analytic accuracy. That is, we somehow have managed to avoid the problems posed by laboratory error and we are testing directly for the gene in question, not a closely linked marker. Even in this ideal case, interpreting the results of the test in a way that is meaningful to patients will be extremely difficult for a long time to come. Granted, the mismatch between analytic and diagnostic accuracy likely will become less severe as our knowledge of the etiology of disease grows. This makes it no less serious an issue at present, however. Moreover, the information required to resolve the problem will be far more difficult to collect than many currently realize.

At its most basic, the reason for this can be stated as follows: The presence of a particular gene associated with a medical condition does not ensure that one will develop the condition (8, 9). There are three possible explanations for this lack of "genetic determinism": multiple mutations (alleles) for a single gene, multiple genes, and gene-environment interactions. If the genetic basis of a condition has many alleles and their frequencies vary widely between populations, then the type of test required and the interpretation of its results must be related in complex ways to the population from which the patient comes. If the condition has multiple genes, then we need to determine all or most of the genes involved and test for each of them simultaneously to obtain an accurate diagnosis. Finally, if the condition exhibits significant gene-environment interaction, as with most complex medical conditions and particularly those involving "propensity genes" (e.g., cancer, coronary disease, etc.), we must be able to assess the variation of significant environmental factors within the patient population to be screened to make diagnostic predictions.

It is important to remember that this is more than the obvious point that, "one can never predict things with absolute certainty." With current techniques, it is very difficult even to determine the probability that the condition being screened for will arise, given the current state of our knowledge about the etiology of most genetic conditions. This is troublesome because supporting accurate diagnoses seems a minimum requirement to justify testing. One way to illustrate the problem is by looking at the procedure most often used initially to isolate a gene associated with a medical condition. Because such conditions are typically quite rare, the first step is to locate a population in which the condition is unusually common so that we have more of a phenomenon to study. Then, researchers conduct statistical analyses to see if there are any regions of the chromosomes which seem to covary significantly with the condition, that is, if there are any pieces of genetic material one is significantly more likely to have if one has the condition than if one is healthy. Once we have located a segment of DNA that appears to covary with the condition, we can use molecular techniques to refine the analysis. In the ideal case, these efforts will isolate a single gene which is then designated as "the gene for" the condition. At this point, we can develop genetic tests to determine whether individuals have the gene, but can we make accurate diagnostic predictions about the likelihood of their developing the condition?

For example, suppose I find that there is a very high incidence of breast cancer associated with a particular gene (BRCA1) in populations of Eastern European (Ashkenazi) Jews. Of Ashkenazi women with BRCA1, 85% will develop breast or ovarian cancer in their lifetimes. Now suppose I produce a test for BRCA1 and administer it to women in the general population. If a woman who is neither Jewish nor European tests positive for BRCA1, do I tell her that she has an 85% chance of contracting cancer? Such a prediction is justifiable only if one of two assumptions is correct: 1) There are no other factors besides the BRCA1 gene which significantly alter the likelihood of developing cancer 2) There are other factors which significantly alter the likelihood of developing cancer, but these have the same distribution in the general population as they do in the Ashkenazi population and therefore do not differentially affect the likelihood of developing the condition from one population to the other.

The first assumption is almost certainly false for a condition like cancer which probably involves multiple genes with multiple alleles and complex gene-environment interactions all at once. So, is the second assumption true? If we are honest, we should admit that we really don't know; both because we do not know all the causal factors which influence cancer and because, even if we did know them all, we have not measured their distribution within the general population. Moreover, the population in which we conducted the initial study was attractive precisely because it was not representative; in particular, it had an unusually high incidence of the rare disorder and associated gene(s). But the same factors which produced this biased distribution in the study population (e.g., inbreeding) are almost certain to have skewed distributions of other factors as well. So the study population is unlikely to have a "normal" distribution of whatever additional factors may be causally influencing the condition, whether they are genetic, cytoplasmic, or environmental. The diagnostic accuracy of the test with respect to the general population entirely depends on the questionable assumption of a uniform distribution of causal factors and is thus suspect, despite the fact that we have (ex hypothesi) a test with perfect analytic accuracy.

Cystic Fibrosis as a Case Study

Cystic Fibrosis (CF) makes a good case study for the type of problem I want to highlight for several reasons. First, it has a relatively simple etiology as genetic conditions go because there seems to be just a single gene involved. Second, it is relatively common, about 1 of every 2,500 whites of European descent is affected. The exact incidence figures cited varies from 1/4700 to 1/1000 because different authors examine subtly different populations (10). Last, it is one of the few genetic conditions for which there are data on the population distribution of the alleles involved (as well as the effectiveness of educational programs).

CF is a condition in which thickened secretions in the respiratory, digestive, and reproductive systems result in chronic respiratory infections, loss of respiratory function, digestive difficulties, and infertility. It can be an extremely debilitating condition and is often fatal, although the median survival age has now climbed to 40 years and approximately one half of CF patients survive into their fifties (with intensive therapy). It is caused by various recessive autosomal mutations in the gene which codes for the cystic fibrosis transmembrane conductance regulator (CFTR) protein, of which there are more than 300 alleles (10, 11). Because most mutant alleles are carried by people who are heterozygous (and thus don't exhibit the recessive disease), as many as 1 in every 30 U.S. citizens (3% of the general population) may carry at least a single copy of a CF mutation.

CF can be diagnosed in three different ways: through an assessment of symptomatology/physiologic performance, through the sweat chloride test, and through genetic testing for a known mutant allele. Because 85% of CF cases occur in families with no known history of the condition, it has been proposed as a desirable candidate for generalized screening of the population (12, 13).

As shown in Table 1, however, almost every combination of cystic fibrosis indicators has been documented. This raises interesting questions about the nature of a disease (14). There is also no simple relationship between particular genetic alleles and the severity of the disease (11, 15, 16). Thus, on the basis of genetic tests and/or sweat chloride values, it is very difficult to predict whether patients will manifest the condition clinically and how severely they will be affected. Last, there are large differences between populations with respect to the relative frequency of the alleles, and quite different screens often are required for different populations. Even for what most people intuitively consider a homogenous population, white Europeans, genetic diversity is the rule rather than the exception. For example, screening for just four alleles in Britain is sufficient to detect 85% of CF cases, but in southern Europe, screening for as many as 60 mutations detects only 75% of the CF cases (16, 17).

Therefore, even for a "single-gene" condition like CF, it is not enough simply to identify all the alleles that are associated with the condition. We also must assess their distribution in various

TABLE 1
Permutations of Diagnostic Criteria in Cystic Fibrosis

Classic Symptoms	Sweat Test	Genetic Test	Status
Positive	Positive	Positive	Classic CF
Positive	Positive	Negative	Occurs
Positive	Negative	Positive	Common
Positive	Negative	Negative	Common
Negative	Positive	Positive	Occurs
Negative	Positive	Negative	Occurs
Negative	Negative	Positive	?
Negative	Negative	Negative	Health

populations and reliably determine into which population a patient should be classed. Note that this last task is not a simple matter of patient self-identification: as anyone who has done family history research knows, people rarely have very clear ideas about their ancestry more than two or three generations back. What is worse, they often have incorrect ideas. All three tasks present complex problems which require a great deal more work to solve adequately.

The Psychology of Information

Above and beyond the information problems discussed above, additional problems occur with the way the genetic results will be perceived. There is a strong psychological tendency to view numerical information as being highly accurate, despite the fact that the actual information content of such numbers can range from 0 (completely uninformative) to 1 (perfect predictor). In genetic screening, the information content of numerical diagnostic predictions is typically unknown or, at the very least, subject to debate. To be sure, this is not a problem limited to the lay public, because even professional logicians and statisticians often make elementary errors in interpreting information, particularly statistical information (18).

In the context of genetic screening, however, this problem looms particularly large. We want patients to be actively involved with their own treatment and require informed consent for any medical procedure done to a patient that might inflict harm. The ideal case is for patients to assess the risks and benefits for themselves and to decide whether to undergo the procedures. To what extent can this ideal be met in the case of genetic screening?

The first question is, "Can the patient be made to understand the relevant details of a highly technical procedure like genetic screening?" The evidence suggests that, with a great deal of effort by trained genetic counselors, they can (19). This is encouraging, although it must be remembered that there is currently a severe shortage of trained counselors. Thus, if screening becomes commonplace, it will be performed increasingly by general practitioners whose ability to act as genetic counselors is in serious question (20).

The next question is, "Are people able to retain the information accurately beyond the immediate context of testing?" Here, the evidence is less encouraging. In one three-year follow-up to large-scale CF screening, 20% of those identified as carriers incorrectly recalled the results of the testing. Moreover, of those who correctly recalled the result, 46% interpreted it incorrectly as meaning that they were only likely to be carriers (19). It has been suggested that this problem may be correctable ultimately by changes in the educational system to produce graduates who are better informed about genetics and statistics; it has even been argued that, on certain conceptions of autonomy, patients have an obligation to thoroughly inform themselves about testing (21). However, such considerations are of little immediate use.

The last question is, "Do people actually want the tests?" The answer to this is complex. Offers of free genetic screening to members of the general population typically result in very low response rates, which suggests that many people have little desire to know their genetic status (22). On the other hand, 70% of those offered screening in person by a health professional accept (6). Whether a patient accepts a test depends strongly on the mode of presentation (4), and this raises the issue of whether even the offer of genetic testing by a physician is, in fact, a neutral act. It is likely that the patient will perceive such an offer as constituting at least an indication of safety and accuracy, and perhaps even an endorsement of value. If so, this will strengthen significantly the perception that the resulting diagnostic prediction is highly accurate.

Genetic Screening and Reproductive Technology

Prenatal Screening

One of the most obvious and compelling but also most controversial applications of genetic screening is its use in prenatal care. It is compelling because we are deeply concerned with the health of our children, often more so than with our own (as any pediatrician can testify). It is controversial because, although there is always the hope that early detection will allow early intervention, this is currently quite rare. For example, there is no conclusive evidence that CF screening enables effective early intervention, despite much early optimism (7). Thus the main outcome of a "bad" genetic result is termination of the pregnancy.

Two techniques of obtaining tissue samples for genetic analysis currently are used widely. Amniocentesis is performed at 14 to 16 weeks of gestation and involves needle aspiration of amniotic fluid containing fetal cells. The cells are then cultured and their DNA analyzed. Chorionic Villus Sampling (CVS) is performed at 9 to 12 weeks gestation and involves collection of cells from the chorion (either by catheter or needle), which then can be analyzed immediately. CVS is a major improvement over amniocentesis because the procedure can be performed much earlier and the results obtained more quickly (24 hours as opposed to 10 to 14 days).

Both procedures are highly invasive and carry a small but significant risk to the fetus (approximately 1% risk of fetal loss). Moreover, some conditions currently cannot be detected at 8 weeks (e.g., some neural tube defects). For these reasons, it is unlikely that genetic screening using these tissue collection techniques will ever become standard procedure for the vast majority of pregnancies. However, this is likely to change soon as new techniques for isolating fetal cells from maternal blood samples come on line (23). It is now possible to detect and isolate fetal cells in the maternal blood as early as 9 weeks gestation.

As little as 15 years ago, it would have been impossible to perform genetic analysis on the vanishingly small amounts of DNA obtained from such a tiny sample of cells, but the newly developed polymerase chain reaction (PCR) technique is capable of amplifying DNA from a single cell quickly and accurately. Moreover, as the Human Genome Project reaches completion and more and more genes are identified, it will be possible to screen very early fetal cells for their genetic contents. In practice, this means that soon there will be techniques that are available very early in pregnancy and are no more invasive or dangerous than a routine blood sample. Under these circumstances, it seems very likely that they quickly will become an accepted part of standard care.

Pre-implantation Screening

With in vitro fertilization (IVF), it is now possible to test extremely early embryos for genetic conditions. In IVF, multiple donated eggs are collected and mixed with donated semen in vitro. It is possible to remove a single cell at the 4- or 8-cell stage of the resulting embryo and, using PCR, amplify and analyze its genes. In fact, it is possible to detect some genetic abnormalities in a single-celled zygote using fluorescent in situ hybridization (FISH), which involves treating the cell with fluorescent genetic probes that will hybridize to some kinds of genetic abnormalities and make the deformity visible under a microscope.

Although early detection is a major advantage, IVF is also extremely invasive. The costs, both emotional and economic, are quite high, with success rates of approximately 14% per procedure and costs of $15,000 (2). IVF preimplantation screening thus will remain a specialized procedure for the present.

Prefertilization Screening

Techniques for the screening of individual gametes are currently being developed. Techniques like FISH will eventually make it possible to certify samples of sperm or eggs as "defect free" and would constitute the ultimate in screening. For one thing, it offers advantages over screening of the parents because, even if both parents are carriers of a recessive condition, there is only a 25% chance the child will exhibit the condition (and a 50% chance the child also will be a carrier). For another, it avoids the issue of termination of pregnancy because undesirable pregnancies will not even be started. At the very least, the technique is likely to become popular with sperm donated to sperm banks because the collection methods are simple and artificial insemination is less invasive, cheaper, and more effective than IVF. Intracytoplasmic Sperm Injection (ICSI) even makes screening of individual sperm before fertilization a possibility.

Some Tentative Conclusions

Healthcare decisions in this country all too often seem to result from an ad hoc synergy of special interests, without adequate consideration of what is in the best interests of either the individual or the community (5, 6). Given the public interest in testing and the enormous amount of money to be made from generalized screening, it is often argued that the advent of routine genetic screens is simply a matter of time (24). Despite calls from several professional organizations that genetic screening should be preceded by careful pilot studies (2), the funding for these studies is difficult to find. The CF follow-up studies were commissioned only because of direction from a special committee of the working group on Ethical, Legal and Social Issues (ELSI) of the National Human Genome Research Institute (NHGRI), itself a highly unusual and perhaps temporary entity.

To summarize, I have tried to establish the following points which must be considered when discussing genetic screening of the general population: 1) Currently, the data that we need to assess the diagnostic accuracy for most genetic conditions is simply not available. Moreover, it is difficult to secure the funding needed for such studies. 2) Nevertheless, members of the general public will perceive testing as diagnostically accurate, particularly if tests are offered as a routine part of care by their physician. Subsequently, this will drive the development of more and more genetic tests by private companies interested in the enormous profits to be had from screening of the general public. 3) It is very difficult (although not impossible) to educate people about the implications of genetic results, especially given the current dearth of trained counselors. It is even more difficult to ensure that they retain the information for the extended periods of time often necessary to make appropriate lifestyle or treatment decisions.

So, what can we conclude about genetic screening of the general population? I would like to offer some tentative conclusions based on the two notions of accuracy I have distinguished.

- Individual patients are simply not in a position to evaluate the diagnostic import of genetic tests. As individuals, they cannot assess population-level phenomena, and as nonexperts, they cannot be expected to comprehend all the technical minutiae. The situation seems analogous to the public release of new drugs: patients might be interested in trying a new drug but we typically do not allow this until we have had a chance to carefully assess its effectiveness and possible side effects. Genetic screening of the general population of adults should fall in the same category. To be sure, there will be special situations; for people in high-risk populations, we have more data on the distribution of causal factors and thus a better grasp of the diagnostic accuracy. Moreover, a total ban on screening would prevent the collection of precisely the data we need to evaluate its effectiveness. However, to the extent that we allow screening of members of the population not known to be at high risk, it should be for the purpose of data collection only and they should be treated as any other experimental subject.
- In cases of prenatal screening, the same information problems apply as with the general adult population. However, to the extent that early intervention may be possible, experimental evaluation of early treatments for genetic conditions certainly is justified. Moreover, allowing limited screening of fetuses may be a good way to generate the data needed to assess the diagnostic accuracy of the screens. Of course, this would require extensive follow-up after the initial screen to determine which children actually developed the condition and which did not.
- Preimplantation and prefertilization screening pose much less severe problems with respect to diagnostic inaccuracy than those associated with other screens of the general population, because a selection often must be made as to which embryo(s) to implant or which gamete(s) to use. If a decision such as this cannot be avoided, and if there is no alternative means of selection which is more informative, it is less problematic to rely on information of uncertain diagnostic accuracy provided by current genetic screening techniques. The decision per se does not have to be justified on the basis of the information, because a decision of some sort is unavoidable. Of course, the precise form of the decision is justified via the genetic information, but the alternative often is to use either arbitrary or morally questionable criteria (e.g., sex selection). Therefore, even if the genetic screening turns out to be completely uninformative (which is unlikely), it is hard to see how we are any worse off using it in these cases.

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Ethical Issues Involving Multifetal Pregnancies

RICHARD L. BERKOWITZ, M.D.

Abstract

Selective termination is a procedure in which an anomalous fetus in a multifetal pregnancy is terminated with the intent of allowing the pregnancy to continue, so that the patient delivers one or more healthy infants at term. Multifetal pregnancy reduction is the elective reduction of three or more fetuses to a smaller number in an attempt to reduce the incidence of premature delivery in these patients. The ethical issues relating to these two procedures are quite different. Data are presented relating to the efficacy and safety of each procedure, and the ethical issues relating to them are discussed. **Key Words:** Selective termination, multifetal pregnancy reduction, ethics

Multifetal Gestations

ONE OF THE IMPORTANT CONSEQUENCES which has accompanied the success of Assisted Reproductive Technology is an increase in the number of multifetal gestations. In a comparison of U.S. National Birth Certificate Data from 1978 and 1988, Dr. Barbara Luke noted that although the number of singletons increased by 17%, the twins increased by 33%, and the number of triplets doubled (1). The increase in quadruplets and higher order multiple pregnancies must have been larger, but this was not reported. These data are now almost 10 years old, and if we had the 1997 data to compare with the 1988 data, we probably would see even more dramatic increases. There is literally an epidemic of higher order multiple pregnancies, and this has created several problems for obstetricians, neonatologists, and the prospective patients who have conceived these pregnancies. I would like to focus on two of these problems because they have very real, and very different, ethical implications.

Let us begin by discussing the case of a woman who was pregnant with triplets. On a screening ultrasound examination performed at 18 weeks, one of her fetuses was noted to have an increased nuchal pad behind its head, a finding which is known to be associated with some chromosomal abnormalities. Because of this, the patient underwent amniocentesis which revealed that whereas two of the fetuses had a normal karyotype, the third had Down syndrome. The issue is what to do when a woman has conceived two or more fetuses, one of which is found to be abnormal. The options are to continue the pregnancy and deliver an abnormal neonate, to terminate the entire pregnancy and lose all of the fetuses, or to undergo a procedure which is referred to as "selective termination." Selective termination has been performed by a variety of methods since the late 1970s. It is important to review some relevant outcome data, because the effectiveness and safety of the procedure should be known before considering other issues.

Identical twins with monochorionic placentas have vascular anastomoses in virtually all cases, and this results in an admixture of the two circulations. This situation virtually never exists in dizygotic twins, or in identical twins with dichorionic placentas. The chorionicity of the placenta can be determined by an ultrasound examination,

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Address correspondence to Richard L. Berkowitz, M.D., Professor and Chairman, Department of Obstetrics, Gynecology and Reproductive Science, Mount Sinai School of Medicine, Box 1171, 1 East 100th Street, New York, NY 10029.

and it is important to do this. If twins have a shared circulation, and one is selectively terminated, there is a very high likelihood that the remaining fetus will die within hours of the procedure. This probably occurs as a result of exsanguination of the living fetus into the circulation of its dead sibling. The remainder of this discussion will be limited to fetuses that have been documented to have a dichorionic placenta on ultrasound examination, because the presence of monochorionic membranes is a contraindication to the procedure.

Selective Termination

Selective terminations of abnormal fetuses in a multifetal pregnancy have been performed at The Mount Sinai Hospital since 1984. When we began, we tried to determine the best way to perform these procedures. During that period, we did seven procedures using a variety of techniques, and the outcomes in those cases were absolutely dreadful. Four of the pregnancies were lost within 3 weeks of the procedure, and only three resulted in the delivery of a healthy baby. We then found that the safest way to perform the procedure successfully was to inject potassium chloride, under direct ultrasound visualization, directly into the circulatory system of the abnormal fetus. Since adopting this approach, we have done 100 procedures in patients whose pregnancies have been completed (2). Four of those 100 patients lost their entire pregnancies, for an overall rate of 4%. The other 96% of the cases resulted in the delivery of one or more healthy babies. In addition, at least one of the losses definitely was not related to the procedure itself. Of the 100 selective terminations performed with potassium chloride, 91 were twins reduced to a singleton. Eight were triplets, six of whom were reduced to twins because one fetus was abnormal, and the other two were reduced to singletons because the abnormality was conjoined twins. There was one patient with quadruplets who was reduced to triplets.

Most of the patients in our series underwent their procedures after 17 weeks of gestation. In New York State, a pregnancy can be electively terminated up to 24 weeks. The reason so many of these cases were done after 17 weeks is that a selective termination cannot be performed until an abnormality is detected. This usually is accomplished either by an ultrasound examination performed at approximately 18 weeks, or after receiving the results of a karyotype analysis after an amniocentesis. The latter procedure is often done at 14 to 15 weeks, although it is still common to obtain the results of amniocentesis at 17 weeks or later. For these reasons, 84% of the selective termination procedures in our series were performed relatively late in the second trimester. A small number of procedures were performed earlier based on data obtained from chorionic villus sampling, which generally can be done between 10 and 12 weeks of gestation.

The overall outcome of patients undergoing selective termination in our series was excellent. The mean age of delivery was 36.8 weeks (full term is anything beyond 37 weeks), so these women delivered relatively late in the third trimester. More than 85% of the patients delivered at 32 weeks or later, and only 14 delivered before that time. All the neonates were healthy and without anomalies.

In the Mount Sinai series, selective termination was performed for numerous anomalies, but the abnormality diagnosed most frequently was Down syndrome. With rare exceptions, a selective termination is not offered in situations where the fetal anomaly is lethal. If nature is going to cause the fetus to die in utero, or very shortly after birth, we don't want to subject the patient to an invasive procedure that carries with it at least some risk to the other fetus(es). An exception is made if there is something wrong with the abnormal fetus that could adversely affect the normal fetus. For example, in some cases of anencephaly, the major disruption of the central nervous system adversely affects the fetus' ability to swallow, which in turn may increase the amount of amniotic fluid in its sac and lead to an increased risk of preterm labor and delivery. This obviously could jeopardize the healthy fetus. There may also be other reasons for selectively terminating a fetus with a lethal anomaly. An extremely religious couple told us that if they delivered a living baby who died shortly after birth, a naming ceremony and funeral would be necessary. They found these events too painful to contemplate, and although they fully understood that they were putting the other fetus at some minimal risk, they felt that this was warranted in their case.

Of the eight triplet pregnancies, the only case that ultimately resulted in loss of the entire pregnancy was one in which conjoined twins were present. That loss occurred about 4 weeks after the procedure had been performed. All the other procedures resulted in the birth of babies of very respectable gestational ages. In the patient with quadruplets, an anencephalic fetus was selectively terminated at 16 weeks, and one of the surviving triplets died in utero at 29 weeks. The patient eventually delivered a set of healthy twins at 36 weeks. I think these results indicate that it is reasonable to offer selective termination to patients with one or more anomalous fetuses. The primary reason is that it works. The procedure accomplishes its objective, which is the birth of one or more healthy babies, and has very acceptable morbidity and loss rates. It generally is believed that approximately 3% of women whose pregnancies progress into the second trimester will miscarry spontaneously before delivery. That figure is not significantly different than the 4% loss rate in this series. A second consideration is that women who undergo selective termination generally deliver their babies at excellent gestational ages, and in our series, the women who underwent this procedure successfully actually had a lower rate of premature delivery than that associated with the number of fetuses they initially conceived.

If one acknowledges that the rates of pregnancy loss, preterm delivery, and neonatal morbidity in patients who undergo selective termination are acceptably low, consideration can be given to the issue of whether or not it is ethically permissible to terminate an abnormal fetus in a multiple gestation. Our laws appear to sanction this concept, because a woman who is carrying an anomalous singleton is permitted to terminate her pregnancy. Indeed, a woman in the United States is given the right to electively terminate her pregnancy even if the fetus doesn't have an anomaly. Therefore, selective termination simply extends this right to a woman who is carrying more than one fetus. Furthermore, the rationale for selectively terminating one abnormal twin, rather than ending the entire pregnancy, is specifically to save and sustain the life of the other fetus. Assuming that the procedure offers no significant risk to either the mother or any other fetus in the uterus, why should terminating an abnormal twin be any different than terminating an abnormal singleton? Of course, people who believe that it is unethical to abort a fetus under any circumstances will not accept the notion of selective termination, but that brings us to the extraordinarily murky heart of the abortion controversy, and is beyond the scope of this paper.

Multifetal Pregnancy Reduction

There is another problem which has been created inadvertently by the success of our infertility colleagues. I refer to those cases in which women conceive an extremely large number of fetuses, up to as many as 12. The most significant problem affecting women who conceive three or more fetuses is their potential for early delivery. The risk of premature delivery is directly proportional to the number of fetuses in the uterus. In most cases, a woman carrying twins will deliver earlier than one with a singleton, triplets earlier than twins, et cetera. If a woman delivers prematurely, there are three possible outcomes: she could deliver late enough for all the babies to do beautifully; she could deliver so early that none of the neonates survives; she could deliver babies mature enough to survive, but so premature that they are damaged irreversibly as a result of the sequelae of severe prematurity. Furthermore, when a woman carrying three or more fetuses delivers, she almost always delivers all of her babies at the same time. Imagine, having not one or two, but three, four, five or more babies in a Neonatal Intensive Care Unit, every one of whom eventually can be discharged with irreversible brain damage or blindness, and/or suffer from chronic seizure disorders or short gut syndrome. All of these are debilitating lifelong handicaps affecting not just one baby, but possibly several. This fear is what is so terribly worrisome to some parents when they learn that they have conceived three or more fetuses.

The procedure that has been devised to deal with this problem is called "multifetal pregnancy reduction." As with selective termination, it is performed under ultrasound guidance, and potassium chloride is injected into the thoracic cavity of one or more of the fetuses. The objective of the procedure is to physically reduce the number of fetuses in utero to decrease the risk of preterm delivery. Even if the procedure is technically successful, there can be no guarantee that the patient will deliver at or near term, because women who initially conceive only one fetus can deliver long before their due date. What we hope to accomplish, however, is to reduce the odds of that happening.

We currently have done more than 800 multifetal pregnancy reductions at The Mount Sinai Hospital, which is an indication of how very widespread the problem of high order multifetal pregnancies has become. To put this in prospective, a study published in 1985 described the outcome of all the cases of triplets delivered during a 37-year period at the University of Iowa Medical Center, which is the tertiary referral center for that entire state. There were 20 patients in that series (3). We have seen more than 800 cases of three or more fetuses during a period of 11 years in one institution in New York City alone.

Most of the patients in the first 400 cases performed at our institution presented with either triplets or quadruplets, but we also saw 50 cases of women who came to us with five or more fetuses (4). The overwhelming majority of these patients were reduced to twins, but 11 were reduced to triplets, and 21 to singletons. Most of the latter procedures were done for medical reasons, but some were elective. Of the 400 women in this series, 368 had their pregnancies progress beyond 24 weeks, which is considered to be the threshold of viability. Infants can survive at less than 24 weeks, but that is very rare. Thirty-two (8%) lost their pregnancies at or before 24 weeks. Thirteen fetuses (nearly 4%) died in utero many weeks after the procedure, and this figure is similar to the number of natural losses one expects in women carrying twins into the second trimester. These single deaths in utero were not thought to be related to the procedure in any case. Seven babies died during the first week of life, and one died at 10 months of age as a result of complications from severe prematurity. All the other infants did well.

The overwhelming majority of women in our series were infertility patients who diligently had been trying to get pregnant, so they were concerned particularly about the possibility of losing their entire pregnancy as a result of the procedure. In fact, most of them were very unhappy about having to consider undergoing the procedure at all. A statistic universally requested by these patients was the procedure-related loss rate. Only 4 of the 32 cases in which the entire pregnancy was lost occurred within 4 weeks of the procedure, 11 occurred 4 to 8 weeks later, and 17 occurred more than 2 months after the procedure had been performed. The greater the interval between the procedure and the pregnancy loss, the harder it is to know if these two things are related. An important question to be answered is how many of these pregnancies would have been lost if the patient had not undergone the procedure. That information is difficult to determine at the present time. In an Israeli series published in 1994, 140 cases of triplets were all diagnosed before 9 weeks by an ultrasound examination which showed that each of the fetuses was alive and surrounded by a normal amount of amniotic fluid (5). Of these 140 patients, 106 continued with their pregnancy without undergoing a reduction procedure, and their loss rate up to 24 weeks was almost 21%. The other 34 patients underwent a reduction from three to two fetuses, and their loss rate was 8.4%, which is almost identical with the loss rate in our series. This is only one study, and the total number of patients is fairly small, but the data suggest that the background loss rate in women who conceive multiple gestations after infertility therapy is quite high and that undergoing a reduction may actually reduce that loss rate. There are theoretical reasons why that might be true. We estimate that only 1% in our 8% loss rate was caused by the procedure, and the other 7% were losses that would have occurred in any event. Nevertheless, the important figure to remember, for patients who are considering this procedure, is that the overall loss rate is 8%.

What are the chances of delivering prematurely if a woman carrying triplets does not miscarry before 24 weeks? In several published studies, the outcomes of a total of 707 such patients have been reported (4). The mean age of delivery for these women was 33½ weeks. Ninety percent of them delivered at less than 37 weeks, 25% delivered at less than 32 weeks, and 7 to 14% delivered between 24 and 28 weeks. This latter period is the worst window in which to give birth because it is the time when the risk is greatest for having babies who develop severe and irreversible morbidity, yet survive. The published data on 103 women carrying quadruplets who progressed beyond 24 weeks indicates that their mean age of delivery was 31 weeks (4). Virtually all these women delivered at less than 37 weeks, half of them at less than 32 weeks, and 10 to 33% between 24 and 28 weeks. In comparison, the 368 women in our series who underwent multifetal pregnancy reduction, and did not miscarry before 24 weeks, had a mean age of delivery of 35½ weeks, which is precisely what one would expect for a population of patients with twins. Eighty-eight percent of these patients delivered at 32 weeks or later, 9% between 28 and 32 weeks, and only 3% between 24 and 28 weeks. These data show fairly convincingly that the procedure does what it is supposed to do. It increases the mean age of delivery for these patients, and reduces their chances of delivering very prematurely. It doesn't eliminate completely the potential for very early delivery, but the odds of that occurring are diminished greatly.

The Potential Costs of Multifetal Pregnancies

In 1994, Callendar et al. (6) examined the hospital costs associated with one hospital's experience with multifetal pregnancies in 1991. Thirteen high order multiple pregnancies and 21 sets of twins were compared with 100 randomly chosen singletons. In comparison with the singletons, the hospital costs for maternal care in the higher order multiple pregnancies was approximately 3 times higher per pregnancy, and the neonatal costs were 6 times higher per neonate. The total cost per family was approximately 11 times higher, and the total cost per baby was about 4 times higher. If each of these higher order multiple pregnancies had been a singleton, the savings would have been more than three million dollars in that one hospital in that one year. Think about how many hospitals in the United States have obstetrical services. Furthermore, some of the items not included in these data were: the charges for neonatal transfer to step-down facilities, the physician fees for the Neonatal Intensive Care Unit or maternal care, the charges for in home neonatal care, the charges for infants with disability secondary to preterm delivery, the economic impact of neonatal mortality (6% for twins, and almost 14% for the higher order pregnancies), the charges for infertility therapy, the charges for outpatient management of a high-risk pregnancy, and the loss of time from work for bed rest, either at home or in the hospital. Therefore, the cost enumerated in this article only represents the tip of the iceberg. The economic impact of these higher order multiple pregnancies on a national level is astronomical.

Ethical Considerations

Let us now consider some of the ethical issues raised by multifetal reduction. When this procedure is performed, one or more completely normal fetuses are electively terminated in an attempt to achieve a theoretical advantage for the remaining fetuses. Ethically, this is very different from terminating a fetus that is known to have a serious anomaly. The ethical quandary here has been likened to a crowded life boat. After the loss of a vessel at sea, when a life boat is absolutely full, but some passengers remain in the water, a decision must be made about whether it is appropriate to refuse admission to one or more people who are in danger of drowning. In that situation, if additional passengers are brought on board, the life boat might sink and the lives of all of those in the boat may be lost. On the other hand, refusing to lift someone out of the ocean may very well be a condemnation to death for that individual. The decision to undergo a multifetal pregnancy reduction is usually fairly easy for women who have conceived six or more fetuses, because, to my knowledge, there have been only two pregnancies with sextuplets and one with septuplets that have resulted in survival of all the neonates. In this situation, the odds in favor of a reasonable outcome are quite good if the procedure is performed, and are terrible if it is not. Virtually no morbidity or mortality data have been published for quintuplets. However, most women who conceive five fetuses know that the chances of their delivering very early are extremely high. For quadruplets, the data in support of undergoing the procedure are fairly convincing, at least when one considers Neonatal Intensive Care Unit admissions and the possibility of irreversible sequelae to infants born at less than 30 weeks. The decision for women carrying triplets, however, is considerably more difficult. There are many women who deliver triplets at very respectable gestational ages, and whose babies have absolutely no neonatal problems. However, a significant number of patients carrying triplets will end up on the wrong end of the Gaussian curve and will have to cope with the potential of having three babies who suffer serious sequelae associated with their very premature birth. This is a gamble that many couples who have conceived triplets are unwilling to take. Please remember that the rationale for undertaking this procedure is to improve the quality of life for the fetuses that have been left alone. This, however, is only a theoretical consideration at the time the decision must be made, because none of the fetuses is in trouble at that time, and their gestational age at delivery cannot be predicted.

Opinions about the ethical acceptability of multifetal pregnancy reduction vary more widely than those relating to selective termination. Those who believe that abortion is wrong under any circumstances obviously will be opposed to the procedure, unless they limit their focus to cases of eight or more fetuses, where clearly two would have a chance to live if a reduction was performed, and virtually none would have any hope to survive without it. The acceptability of the procedure becomes more controversial when smaller starting numbers of fetuses are considered. Some physicians, convinced that existing literature supports the conclusion that multifetal pregnancy reduction is likely to increase the quality of life for the survivors in pregnancies with three or more fetuses, justify the procedure as being medically indicated in those cases. Others wlll perform this procedure electively on women with twins who either don't want to give birth to two babies or hope to marginally increase their chances of delivering a singleton at a later gestational age. In these situations, the procedure has very little medical justification, but its supporters argue that if singletons can be aborted electively, it should be permissible to electively reduce twins to singletons. Again, fundamental sentiments about abortion are often the determinants of how individuals feel about these different positions.

I have tried to address some of the ethical issues associated with multifetal pregnancy reduction as contrasted with selective termination. Certainly the data accumulated at The Mount Sinai Hospital and other institutions which have performed many of these former procedures have given us good reason to believe that it achieves its objective and is safe. Thus, I am comfortable offering patients this option for what I consider to be medical indications. On the other hand, I think the best solution to this problem of high order multiple pregnancies would be not to have them. The overwhelming majority of women who conceive three or more fetuses today are infertility patients. The creation of multiple pregnancies can never be avoided completely with modern infertility care, but the large number of high order multiple pregnancies we currently are seeing is excessive. I look forward to the day when ethical issues relating to multifetal pregnancy reduction are moot because the procedure is not needed. Until that time, however, this procedure is a reasonable option to offer women who conceive three or more fetuses.

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Reproductive Choice and Public Policy:

A Doctor's Perspective

Kurt Hirschhorn, M.D.

The past few decades have seen a vast expansion of reproductive choices available to couples who are at risk for abnormal offspring and who have fertility problems. Because many of the options as well as the diagnostic studies related to these have ethical, legal and religious implications, there has been much public and government interest in the regulation of the technology involved. I will address four of the relevant topics and some of the public policy responses established or contemplated which have an impact on the availability and reality of these choices.

Prenatal diagnosis has been widely available since 1969 and is relatively safe. Currently, the major techniques being used are amniocentesis with analysis of the fetal cells found in amniotic fluid, and chorionic villus sampling which involves a biopsy of the placenta and analysis of the fetal cells found in that organ. These two techniques are well established and subject to no more public regulation than any other medical intervention. The only exception is that they are limited in time, because results are obtained before the end of the second trimester of pregnancy to avoid pregnancy terminations after 24 weeks. Whenever abortion is at issue, there are numerous persons who, for religious or other reasons, wish to interfere with any procedure that may lead to abortion, not only for themselves but for the general population. The most recent debate relating to this question concerns a technique for late abortion that has become a lightning rod for the right-to-life movement which has been attempting to convince Congress to ban this method even in cases where it is necessary for protection of the mother's life and her health. This is a minor disturbance in a well-established technology, however.

A newer method of prenatal diagnosis has been developed and is beginning to be used. This new method, preimplantation diagnosis, generally consists of in vitro fertilization, laboratory development of the embryo to the eight-cell stage, and removal of one or two of these cells for diagnostic analysis. Embryos not affected by the condition for which they are at risk are then implanted into the mother and, in those cases already reported, have resulted in normal offspring (1). Attempts are being made to perform prefertilization diagnosis on ova and possibly sperm, thereby entirely avoiding issues raised by those who consider an eight-cell mass a human life. Although these latter techniques have been devised partly to avoid the controversial issue of abortion, their perfection for more general use has been impeded by the public policy of not permitting Federal funds to be used for embryo research. These policies have been pushed by the same lobbies that wish to abolish abortion. Despite the recommendations of a broadly based panel that research during the first 14 days of embryonic life should not only be permitted but encouraged, especially for a better understanding of normal human development without which many congenital diseases will not be understood, the government has not accepted these recommendations to avoid criticism from a vocal minority (2). These rules have had the effect of forcing some leading practitioners and researchers of preimplantation diagnosis to give up their gov-

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Address correspondence to Kurt Hirschhorn, M.D., Professor of Pediatrics, Human Genetics and Medicine, Box 1497, Mount Sinai School of Medicine, One East 100th Street, New York, NY 10029.

ernment and university positions. In the latter case, they fear that grants to that university may be at risk because of the misinterpretation that preimplantation diagnosis may be considered to be embryo research (3).

The second topic which relates to some of these choices is gene therapy. Virtually everyone agrees that the next few years will bring increasing activity in the clinical application of somatic gene therapy. The special National Institutes of Health (NIH) committee which had been created to oversee both policy and technology in this exciting new field essentially has been abolished and its duties handed over to the Food and Drug Administration (FDA) on the presumption that gene therapy is now just another medical technology. To avoid risky or foolish applications of gene therapy, public oversight is needed, more than is possible by the FDA. Yet, I can understand why the NIH wishes to be rid of the responsibility for looking at every proposed application because many of the experiments will be done without federal funds, and some of the best scientists will be forced to work under severe restrictions. If intrauterine gene therapy becomes available, as is very likely, it would provide another reproductive option for couples at risk for abnormal children. Currently, the major public policy on gene therapy is the taboo on germline genetic correction. It has been demonstrated, at least in the mouse, that germline correction of a genetic defect results in a normal individual and prevents the transmission of the mutant gene to that individual's progeny. It is this fact which has led to the public ban on germline gene therapy because it involves therapy for the individual and has an impact on future generations. Although debates about this procedure continue, for the moment it remains forbidden and appropriately so. This is appropriate, because we first need proper analysis and debate on a national if not an international level. Prenatal gene therapy, or in some cases even postnatal gene therapy, may introduce genetic material into germ cells and inadvertently allow transmission of the corrective or corrected material to future generations. No one talks much about this possibility because it may have an impact on the current enthusiasm for gene therapy and the related loss of interest in its regulation by policy-making bodies.

The third somewhat controversial topic relating to reproductive options is genetic screening. It is already possible to screen different types of populations for different types of genetic risks. Some of these efforts are now quite routine, and public policy either ignores them or allows them to go forward with minimal regulation. These include prenatal screening for neural tube defects as well as chromosomal aberrations and newborn screening for a variety of inborn errors of metabolism. Some inborn errors of metabolism now occur primarily in ethnic groups with a particularly high frequency of specific mutations. These include Tay-Sachs and Gaucher disease in the Ashkenazi Jewish group, thalassemia in Mediterranean, Chinese and African populations, and abnormal hemoglobin in Black and Asian populations. Two developments have led to a great deal of activity in attempting to influence public policy regarding genetic screening. One of these is the current ability to identify some women at high risk for breast cancer because of their inheritance of a powerful susceptibility gene (4). The other is the rapid development of multiplex screening which will enable us to detect rapidly multiple mutations in many genes in numerous persons. The latter will be made possible by the further development of small chips that carry probes for hundreds of mutations which they can detect from a small amount of DNA (5). A few years ago the Institute of Medicine published a volume about genetic screening including controversial conclusions (6). An important feature of the controversy relates to the extent of informed consent for screening. Everyone agrees that before a woman is tested for the breast cancer susceptibility mutations, she must be exposed to a thorough educational discussion as to what a positive or a negative result means for her. A negative result does not rule out the possibility of breast cancer, although a positive result does not, at this time, point toward successful preventive measures. Informed consent becomes more controversial in several other contexts. For example, multiplex screening could be applied to the newborn population to detect rapidly and accurately mutations for disorders in which preventive or therapeutic interventions are helpful. Should parents or prospective parents be permitted to refuse such screening when an affected child could be damaged severely because the disorder is not detected in a timely fashion for therapy to be instituted? Detailed educational discussions about all of the screened mutations and their implications for the child and the family would involve a process that could not be completed until long after the child was born. The staff necessary to obtain such in-depth informed consent for every pregnancy screening is not available. A special committee of the working group on Ethical Legal and Social Issues (ELSI) of the National Human Genome Research Institute (NHGRI) has produced a series of genetic screening guidelines (7). Many geneticists disagree with several points made by this committee, and it remains to be seen how many of their recommendations become public policy. President Clinton recently appointed a Bioethics Commission to consider many of these issues. In addition, the ELSI committee has recommended that another commission in the Office of the Secretary of Health and Human Services (HHS) be formed to deal with the ethical problems of genetic screening. Another committee of NHGRI has recommended yet another national committee in the Office of the Secretary of HHS to deal with ethical and social aspects of the genome project and its outcomes. If all of these commissions are put to work, it is likely that they will produce confusing sets of rules and recommendations for public policy which, therefore, will be followed variably or ignored. Preferably, the government might be persuaded that a single commission with a good deal of public and scientific participation would suffice. Certainly more accurate and more widely applied genetic screening will have a major impact on the choice of reproductive options for couples at risk. Regulations having an impact on screening, therefore, will have a major effect on the ability of couples to make decisions.

The fourth topic I will address has been brought to public attention recently and has led to widespread, almost hysterical fears and public pronouncements. This topic is cloning. It was reported in *Nature* (8) that a lamb was born which was produced by the fusion of an adult sheep's somatic cell with an enucleated ovum from another sheep. What makes this result different from previous work is that the sheep which was cloned was an adult and, therefore, its characteristics were known. It should be remembered that the identical techniques were reported a year ago (9) and in other past work even longer ago in which sheep or other animals were produced by cloning embryonic cells using the same procedure (10). Embryonic cells already have been cloned to produce monkeys. Therefore, there is nothing fundamentally new that should have led to this hysteria except that the ability to clone an adult leads to a variety of fears that specific human adults will be cloned for peculiar purposes or for their characteristics. Clearly, cloning oneself or one's dying child is the ultimate reproductive option. This latest advance rapidly led to a temporary ban on work in human cloning by President Clinton; again this restriction only applies to those who work with federal funds, not private companies or individuals. This work also has led to laws forbidding human cloning in some European countries.

We should be aware of some misconceptions before leaping to the conclusions seen in the newspapers and on television. First, if we clone an adult, the genetic copy of that individual will become an adult a generation later. Because of the major impact of environment on the individual's development and activities, the cloned individual will be very different from the source of the clone, beginning with a different intrauterine environment. What is desirable or attractive in one generation may be different 30 years later. Also, it should be remembered that ova contain many mitochondria which carry a significant amount of DNA, that can determine a number of characteristics passed on by maternal inheritance. Some genetic differences between the original individual and the clone may be imparted by the presence of mitochondrial genes in the ovum used for the clone and these generally will not be derived from the cloned individual. In addition, somatic mutations continuously occur, changing a variety of cells in each individual. Finally, the establishment of networks in the brain leading to specific behavior and activity will differ to a great extent from individual to individual, much of it based on stochastic or accidental events. This is why one finds significant differences between the personalities of identical twins.

Therefore, the expectation that one can make a bunch of Einsteins or the fear that one could make a bunch of submissive slaves is quite unrealistic. Nevertheless, all these possibilities deserve wide discussion, and I generally agree that public policy should be developed to regulate such activities. Also, it must be considered that making several genetically identical individuals, in the long run, will diminish the beneficial heterogeneity of the species, reducing our resistance to environmental and infectious disasters. The same risks are incurred by fertilizing many women with one or a few sperm sources, e.g., from sperm banks established to carry sperm from so-called superior men.

Almost 30 years ago, I was asked to comment on many of the topics discussed here which then were only ideas or hypotheses. In a publication called *On Redoing Man* (11), I came to similar conclusions and suggested that society might think about these technologies before using them, because it is generally true that what can be done will be done. However, as usual, we are only dealing with the implications of these significant proposed interventions now that the reality is upon us.

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The End of Motherhood as We Have Known It

Patricia S. Mann, Ph.D.

Abstract

The new reproductive technologies have dramatic social implications, undermining previous biological assumptions about the relationships between mothers and fathers and children within families. Insofar as we may now distinguish between genetic motherhood, gestational motherhood, and social motherhood, three different women may participate in the decision to produce a child. Who is its "real" mother? Given the complicated interpenetration of nature and technology within reproductive situations today, we need to develop new social accounts of what it means to create a family. We require new narratives that highlight the wondrous kinship possibilities afforded by the new reproductive technologies while at the same time articulating the responsibilities of parenthood within new familial groupings. **Key Words:** Motherhood, families, reproductive technologies

As a social philosopher, I believe that we need to confront the dramatic social implications of our technological capacities. Hannah Arendt famously complained that scientists today "act into nature" rather than "into the web of human relationships," seducing us into a science-based way of life in which we are capable of doing a great many things about which we cannot think or speak adequately (1). In the new reproductive technologies, such a danger is very real. Simple and obvious biological facts become philosophical conundrums with the new technology. For example, when Ian Wilmut, the creator of the cloned sheep, Dolly, was asked how we should compute her age, whether her birth in July makes her a 7-month-old lamb or instead a 6-year-old genetic replica of the sheep from which she was cloned, he said that he couldn't answer that question. "We just don't know," he said (2). And in the much more familiar world of gestational surrogacy, we are now creating children with two biological mothers. When surrogacy agreements go awry and maternal rights are in question, courts must determine which biological mother has best claims upon social motherhood. Asked who the real mother was in any of these cases, a neutral bystander would have to say, "I just don't know." The biologically based web of kinship relationships has been altered fundamentally. How should we even begin to articulate the human significance of this fact?

It's a little daunting to contemplate, but tinkering with a few threads in the fabric of human relationships can lead to a lot of unraveling. I believe that the new reproductive technologies are contributing to a major paradigm shift in our society today involving most immediately the status of women, and only slightly less immediately, the status of families and gendered notions of personhood. We can only make appropriate moral, political and legal decisions regarding the new reproductive technologies insofar as we appreciate these larger social dynamics. Our beliefs regarding the proper applications of reproductive technologies will vary according to the role we envision for them in this larger process of social transformation.

There is a perhaps understandable tendency on the part of many of those officially engaged in articulating the new reproductive issues to play down their most radical implications. Indeed, from expert advocates like John Robertson, to family court judges and lawyers, to genetic coun-

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Address correspondence to Patricia S. Mann, Ph.D., 61 Jane Street, Apt. 14G, New York, NY 10014.

selors, there is an attempt to assimilate even the most radical new procedures into a model of the traditional family and its enhancement. Although critics of the new reproductive technologies are more attuned to the ways in which they will change our lives, such critics tend to posit traditional motherhood or kinship relations as ideals threatened by the new technologies. I have very little sentimental attachment to traditional notions of motherhood or family. I am delighted that ours is an era of great social change, particularly with regard to gender relations. My goal here is to analyze the relationship between these reproductive technologies and what I take to be newly emergent notions of personhood and family.

Part I: The Social Enfranchisement of Women

It is hardly controversial to refer to a dramatic change in women's status during the past 30 years in this country. In an astonishingly rapid shift in social consciousness, we have come to take for granted the workforce participation of most women, as well as women's ability to control their own fertility. Young women and men today have no memory of a time when women were expected to remain at home raising children after marriage, dependent on a husband who was solely responsible for the economic maintenance of the family. We teach about these ancient practices in women's studies classes. Of course, this lifestyle is still an option for some, but only a minority of couples chooses it for any length of time. Indeed, the large-scale workplace participation of women, as well as the use of contraception are now global trends, so are the new rights and responsibilities of women that we associate with these trends. I will refer to the empowerment, or what I call the "social enfranchisement of womem," as an ongoing feature of global transformation.

Women's new economic and reproductive rights and responsibilities undeniably have improved women's status in relation to men. At the same time, they have created many new sites of conflict and confusion. I first became interested in reproductive issues a few years ago in the context of the abortion controversy. I was puzzled by the fact that we can make persuasive political arguments about a woman's right to an abortion, and yet find it very difficult to make compelling arguments for the morality of abortion. I was troubled by the fact that there are many situations today in which society expects a woman to have an abortion, but simultaneously withholding full moral approval.

Judith Jarvis Thomson's famous article, "A Defense of Abortion" (3), highlights this orientation, because it powerfully articulates a woman's right to refuse to do a good deed. There is something perverse about comparing a woman's right to an abortion to her hypothetical right to tell a famous violinist that he must die insofar as she is not willing to share her kidneys with him for 9 months. With this sort of imagery, it is hardly surprising when Thomson concludes that there are circumstances in which we might judge a woman "self-centered and callous, but not unjust" in deciding to have an abortion. When several feminist acquaintances expressed regret over their abortions many years before, I decided to investigate further.

What I found was a traditional conception of maternal agency lurking in the shadows of our social unconscious. We know that women were long seen as part of an organic natural continuum; upon becoming pregnant, a human mother, like any other member of the animal kingdom, had an unqualified responsibility to bear her child. A woman's most basic motivations were presumed to accord with the frequent need for sacrificing her own interests on behalf of those of her children. And a woman could expect to be supported in her maternal role by a kinship structure, her spouse and extended family members sharing the economic and social burdens of raising her children. Women who did not have children, by virtue of infertility or taking religious vows, were not complete women. Motherhood was an identity condition of womanhood. According to such a perspective, a woman who had an abortion would be denying her own nature, as well as Nature with a capital "N". And a woman's infertility would be deemed a cruel turn of fate, tragically robbing her of her birthright as a woman.

Of course, a traditional sense of maternal agency is quite unrealistic today. There is no longer an unqualified social obligation for individual women to produce children. China's one-child policy is only the most extreme example of a new global emphasis on population control. Consequently, individual women have become responsible for using contraception to drastically limit their production of children. In addition, structures of kinship support for motherhood have waned considerably. A woman may often find herself the sole supporter of her children, economically and otherwise. At the same time, we no longer assume that a woman will be or ought to be motivated to sacrifice herself totally on behalf of her husband and children. Acknowledging women's new workforce participation, we assume that a woman's public responsibilities may conflict and compete with whatever maternal responsibilities she may have. Our ideals of equality and fairness require that women be honored for their public achievements, regardless of whether they have fulfilled a maternal role.

In my book, *Micro-Politics* (4), I develop a theory of agency to talk about the different ways in which women and men have been expected to understand their actions, and also to chart the changes in women's and men's notions of agency as women become socially empowered. I suggest that there are three dimensions through which we typically understand the significance of our actions. We act in response to desires and responsibilities, as well as with expectations of recognition and reward. Within the modern world, the dimension of individual desire has been presumed dominant in men, explaining their pursuit of the wide variety of activities open to them. In women, however, the dimension of responsibility, specifically maternal responsibility was presumed to be dominant. In fact, when we looked on a little girl and fondly noted that she would make a wondertul mother one day, we were imposing on her an unconditional responsibility of motherhood. Her desires and expectations of recognition and reward were expected to conform to her maternal obligations.

The social enfranchisement, or empowerment of women, during the past 30 years is associated with a major change in our understanding of female agency. Feminists insisted on the startling proposition that as full human beings, women have desires and capabilities for accomplishing the full array of economic, political, and cultural deeds we formerly associated only with men. Maternal obligations could no longer dominate our sense of women's agency, insofar as women may desire to do and be recognized for many other social roles. Indeed, women's new responsibility to become economically independent meant that they must be recognized for many nonmaternal roles. And women's responsibility to control their fertility meant that motherhood was no longer an unqualified obligation. In their daily lives, women today find themselves with the technical capacity and the social responsibility for making very cautious decisions as to whether and when and how to have children. Maternal agency is no longer an absolute condition of womanhood, as it had been for thousands of years.

But it is not easy to leave the old narratives of motherhood behind. Many women continue to feel implicated in motherhood from the moments of their birth. For one thing, an antiquated aura of moral rectitude continues to surround motherhood. In traditional societies, a mantle of unqualified maternal Goodness functions like a bribe, reconciling a woman to the burden of maternity that she cannot refuse in any case. In our society, this normative privileging of maternity is still operative in various ways, with frequently inappropriate results. Among couples and single women who have devoted themselves single-mindedly to careers and worldly possessions, it suddenly can seem necessary to have a child to fulfill a vague sense of unrequited possibility. Forty-something women who have proven themselves to be successful at every other aspect of life are prone to feelings of inadequacy for not having realized their maternal potential. Childless women of means resort to extensive and expensive fertility treatments with only the faintest hope of finally bearing a child. Given the maternal obsessions of women who have so many other sources of personal affirmation, we should hardly be surprised when teenage girls from impoverished backgrounds imagine that motherhood will provide them with a sorely needed sense of personal significance.

So long as we continue to subscribe to a traditional gender narrative according to which all women are implicated in motherhood at birth, a woman's decision to become a mother will retain its valence of unconditional goodness. Any woman's decision to bear a child will seem fundamentally natural and good, no matter how capricious or inappropriate this decision may be in her actual circumstances.

Part II: Cyborgean Reproductive Agency

In *Micro-Politics* (4), I proposed a cyborgean theory of maternal agency as an alternative to the naturalistic account. I hope that this cyborgean narrative can help us gain an appropriate perspective on an array of reproductive technologies, from contraception to cloning. In science fiction, a cyborg is a "creature simultaneously animal and machine, populating worlds ambiguously natural and crafted," to quote Donna Haraway (5). A cyborgean image of personhood emphasizes the contemporary breakdown in our sense of boundaries between our organic selfhood and the machines we increasingly incorporate within our everyday activities.

Until very recently, we have taken for granted the alien qualities of the machinery and technologies on which we rely, their usefulness always bounded by their metallic, clanking lack of ensoulment. The staunchest advocates of the machine age have continued to stress the need to vigilantly maintain human control over whatever artificial intelligences and robotic wonders we may create. And in the view of critics, from Luddites in the nineteenth century to deep ecologists and radical feminists today, we must fear for technology's ability to ride roughshod over those unique aspects of our humanity that we most value. The recent cloning of Dolly the sheep has brought all these fears and warnings back into public discourse.

Yet it is hard to regard many forms of technology as alien presences in our lives. We awake to the insistent beeping of digital clocks and amiable microwave ovens, slide behind the wheel of a car accompanied by more interactive electronic beeps, and typically maintain intimate aural and physical contact with various technologies throughout a normal day. Moreover, we regularly choose to undergo operations in which our hips, knees, and other organic body parts are removed and replaced with artificial ones. And for most of us who write as a way of life, our personal computer has become our favorite means of accessing our ideas and harnessing our creativity.

We describe our relationships to these machines in very peculiar terms, by earlier standards; but these terms express the degree of technological penetration into our personal lives. We gratefully say that artificial hips and knees make us feel "like our old selves again." And after becoming accustomed to writing on a computer, we may express horror at the idea of returning to previous writing practices which may now seem both less effective and less enjoyable.

Are we still in control? Does our dependency on these machines make us less human? The point of the cyborg image is to suggest that these questions have lost their meaning. These machines make us better able to do the things we value most as individual human beings. Were we to be forced to give them up we would feel diminished as human beings. This does not deny their quality as machines and technologies. It does imply that we need to rethink our relationship to machines quite radically so as to expunge anti-technological biases that have become hypocritical and misleading at this point. Our dualistic Cartesian world view makes us vulnerable to fears that once technology gets a sufficient foothold, the natural world will be absolutely displaced by it. My goal is to show the possibilities for a social mediation of nature and technology that seems already inevitable.

Our relationship with technology is nowhere more intimate or more significant than in our sexual and reproductive lives. Individually, we may rely on various sorts of contraceptive technology on a daily basis for most of our adulthood. When we decide to engage in reproduction, we increasingly call on esoteric forms of reproductive technology as well, whether as a means of enhancing fertility or as a means of ensuring the well-being of fetuses before birth. Reproductive methods that take place partially or wholly outside the womb are developing rapidly, and it perhaps will not be too long before parturition can proceed entirely outside the female body.

There are now clear grounds to cast aside the narrative of women's organic implication in motherhood from birth. Our cyborgean dependence on technology as a normal condition for reproductive decisions transforms women's role in the procreative process. The old organic quality of human reproduction is dominated today by technological procedures and the particular social decisions they make possible and necessary. The complicated interpenetration of our natural and technological capacities should clarify the pivotal role of our social selfhood. The perplexing entanglement of our old organic relationships with postmodern technological ones can only be countered by a clear vision of our particular social situation.

In our cyborgean world, women are procreationally neutral. There is no need for any particular woman to have a child. Children are, of course, very nice things to have in the best circumstances. Some women will want children in almost any possible circumstance; others only in certain circumstances; and others in no circumstances. It is no longer morally sufficient for a woman to explain her decision to have a child by saying that she is a woman. We know that socially there is a problem of overpopulation, and individual women frequently may lack either the desire or the ability to assume parental responsibility for children. An unconditional obligation to become a mother makes no sense at all. For cyborgean female selves, these social realities finally become embodied as lived truths, insofar as the technology of contraception becomes more of a daily reality than the organic possibility of pregnancy.

Insofar as the organic conditions for producing a child are interpenetrated with technological and social considerations, children become products of our social decisions in a thoroughgoing sense. It no longer makes sense to think about children in terms of their natural origins. Children will come to be seen as "social offspring" of many possible procedures and practices in which technology and nature are intermingled inseparably. Our children will remain historically specific children and will surely continue to ask from whence they have come. But our answers will refer to the various social decisions that led to a child's birth. From a cyborgean perspective, social offspring are born of acts of interpersonal decision-making and only incidently of women's wombs.

Part III: Assisted Reproduction and the End of Motherhood as We Have Known It

Insofar as most women rely on technologies of contraception for most of their adult lives, pregnancy and motherhood have ceased to be natural or necessary. It is now mandatory for each individual woman to decide if and when she will have a child or children. Unfortunately, many women resist the cyborgean implications of contraception; when they want to become pregnant, they simply stop contracepting, and they seem to become pregnant quite "naturally."

Only in the 15% of cases when infertility problems appear does the cyborgean/unnatural quality of motherhood, and indeed fatherhood, become patently evident. The pill made sex without reproduction possible, but only the new reproductive technologies make reproduction without sex possible. Indeed, reproduction now typically occurs in a dish or in a tube somewhere at a spacial and temporal remove from these cyborgean genetic parents. Even more remarkably, with techniques such as the cryogenic preservation of embryos and the harvesting of eggs from aborted fetuses, cyborgean parents can be totally absent from this world. And now with cloning, a single cell of a woman or of a man can potentially become the genetic parent of a child. (I have wondered at the symbolism of Dolly's origins in a mammary cell, and have been surprised to see no commentary on this aspect of the event.) In any case, this is truly the end of motherhood as we have known it.

Feminists such as Barbara Katz Rothman have some reasonable worries about the reenforcement of patriarchal values, insofar as the new reproductive technologies emphasize the genetic link rather than the act of female parturition (6). Indeed, Judith Lorber has written about the marital bargaining that goes on in cases of male infertility, and concludes that it may be very difficult for women to say "No" to painful in vitro fertilization (IVF) procedures, for example, in the face of a spouse's desire for a biological heir. She concludes that women counselors in IVF programs may at least be able to help women make "the best possible patriarchal bargain" in this situation (7).

I am hopeful actually that the outrageous unnaturalness of these new reproductive options, the plethora of high-tech macho reproductive gizmos, may finally encourage women to develop the intellectual and emotional distance on motherhood that is already appropriate in a cyborgean age. Traditional motherhood was characterized by absolute female responsibility for childbearing. Cyborgean reproductive agency is about relativizing and limiting that sense of female responsibility. Ironically, a cyborgean moral agent may be freed from the technological imperative even as she accepts the usefulness and desirability of technology in some instances.

Parental agency is no longer a matter of necessity, and it must now be articulated in ways that emphasize its transformation into an act of interpersonal commitment and total reproductive freedom. Hannah Arendt (1, chapter 2) drew a memorable and relevant comparison between ancient Greece and our Modern Age. In Greece, she said, the family was the sphere of human necessity; whereas the polis was the realm in which human freedom was enacted. In the modern world, she said, the political sphere has become a social sphere, focused entirely on necessities of keeping peace, protecting private property and so on. Families have lost their original relation to human necessity, even as the public sphere has ceased to provide a site for the expression of human freedom. Arendt was dismayed both that the family had lost its purpose and that there no longer seemed any appropriate site for the expression of human freedom, which she defines in terms of speech and actions celebrating our human plurality, and done without any particular useful ends in mind.

I want to suggest that for cyborgean individuals, the family should become a site of self-conscious freedom. It is no longer necessary to come together at all as families; women and men are now recognized as economically autonomous individuals. The bonds between women, men, and children are freely chosen, or ought to be. Insofar as these new cyborgean families are creative enactments of human freedom and plurality, the quest for biological connections to children should decline greatly. The intense pursuit of genetic connections that fuels the current demand for IVF and other reproductive technologies will not make sense. The terminology "Assistant Reproductive Technologies" confuses the issue, because it implies that the natural act of procreation simply is being assisted technologically. I have argued that technology and nature are intertwined inextricably. Reproductive technologies have a fundamental role today, as does nature. Genetic connections will become merely an interesting option; they will no longer seem necessary.

I think that the ethical use of new reproductive technologies, from IVF to cloning, depends on our ability to recognize ourselves as cyborgean reproductive agents. I am not sure what the ethical uses of New Reproductive Technologies will be. But attempts to recreate the traditional family are increasingly futile, so that justifications for the use of reproductive technologies that rely on such a goal are misleading. Whatever the moral grounds for using reproductive technologies are determined to be, they will not emphasize desires to create traditional biological families.

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Ideology and Technology:

The Social Context of Procreative Technology

Barbara Katz Rothman, Ph.D.

Abstract

Biomedical practices and research in procreation are shaped by three ideologies deeply rooted in American society: patriarchy with its control of women's bodies in the interest of men's procreative concerns and with its focus on 'seeds' and therefore genetics; technology as an ideology of efficiency, productivity, rationality, and control; and capitalism both as an ideology and as a practice, a mechanism that markets technologies of procreation. **Key Words:** Prenatal care, prenatal testing, reproductive technology, patriarchy, technology, capitalism, feminism

I BELIEVE SOMETHING HAS GONE seriously awry in our cultural understanding of motherhood in America. I find myself surrounded by contradictions. I live in a world that makes no sense. We in the United States have seen the return of the midwife and the rise of the cesarean section. For 50 years, there were almost no midwives to be found in most of America. Then, in the same period that saw the nurse-midwife introduced into American hospitals, that saw the introduction of "birthing rooms" to replace some of our operating-room-like delivery rooms, that saw an enormous "natural childbirth" movement, we also saw the cesarean section rate rise to an astonishing 24%. Almost one of every fourth births in the United States is now by cesarean. Although there has been much talk of birth as a "healthy, natural event," each individual birthing woman has been acquainted with her personal "risk factors," factors that she is told make her birth less than healthy and far less than natural.

Cigarette advertisements, clearly aimed at young women, the fastest growing market for cigarettes, carrying a warning about how smoking harms fetuses. Alcohol companies also have discovered the modern woman as a new market. Bars, beer cans, and wine and liquor bottles now carry warnings about the dangers of alcohol use in pregnancy. The advertising is acknowledged by the government to be a legitimate business expense; the warnings are a government requirement.

The infant formula companies distribute sample packages of formula for new mothers labeled "In support of your decision to breast-feed." Hospitals, where virtually all of our births take place, hire lactation consultants, show breast-feeding videotapes, and send all mothers, including breast-feeding mothers, home with packages of formula ready-packed in disposable bottles.

Although white babies are worth their weight in silver, if not gold, some darker-skinned children linger in foster care for their entire childhood. And however much children may be valued, nurturing and fostering of them is not. I heard an angry black social worker on television say of a grieving white woman whose foster child was taken away after 3 years—the child's first and only 3 years—because the woman was white and the child was black, "She had no right to love that child. It was just a job." And pretty much the same thing is said of a pregnant woman who is not, I am supposed to believe, the mother of the baby in her belly because in a "surrogacy agreement," the baby was contracted to be there.

Address correspondence to Barbara Katz Rothman, Ph.D., Professor of Sociology, Baruch College and the Graduate Center of the City University of New York, 17 Lexington Avenue, New York, NY 10010.

Childbearing at 40 years of age is now considered chic, childbearing at 50 and older is the new frontier of procreative technology, but childbearing at 18 is considered pathetic, chances lost. And we have one of the highest teenage pregnancy rates in the developed world.

In the name of family values, family needs have gone unmet. We are seeing deeper cuts into family services, from day care to maternity leave to universal prenatal care to child welfare payments for desperately poor people, at the same time calling for a return to "family values."

These are the contradictions I live with daily. This is the world in which I study motherhood, mother my children, live my life. To understand it, to explain it, to find some reason in the unreasonable, I have to step back from it and try to disentangle the contradictions. When I do so, I find myself unweaving the strands of a fabric, understanding the pattern as I work it backward to the underlying threads.

I believe that American motherhood rests now on three deeply rooted ideologies that shape what we seen and what we experience (1). These are the three threads which compose the fabric of motherhood in America today: an ideology of patriarchy, an ideology of technology, and an ideology of capitalism. As these three come together, with their multiplicity of meaning, they give us the shape and the discordance of the American experience.

An ideology is the way a group looks at the world, a way of organizing our thinking about the world. An ideology can let us see things, but it can also blind us, close our eyes to our own lived reality, our own experiences, our own bodies. These three ideologies, of patriarchy, technology, and capitalism, give us our vision while they block our view, give us a language for some things while they silence us for others. In what follows, I will speak first of each of these as separate ways of thinking, but what I understand most clearly is the way they weave together to create a pattern, a fabric, both a curtain and a cage.

The Ideology of Patriarchy

The ideology of patriarchy is perhaps the easiest to understand of the three ideologies that shape American motherhood. Women's reality, in the United States as in most of the world, is not the dominant reality, and women's view of the world is overruled by men's view. Motherhood in patriarchal societies is what mothers and babies signify to men. For women throughout the world this has meant too many pregnancies or too few; "trying again" for a son; covering up male infertility; having some of our children called "illegitimate"; not having access to abortions we do want and being pressured into abortions we may not want.

The term "patriarchy" often is used loosely as a synonym for "sexism," or to refer to any system where men rule. The term technically means "rule of fathers," and it is in that sense that I use it. Male dominance and patriarchal rule are not quite the same thing, and when the subject is motherhood, the difference is important.

Patriarchal kinship is the core of what is meant by patriarchy, the idea that paternity is the central social relationship. A very clear statement of patriarchal kinship is found in the book of Genesis, in the "begats": Each man, from Adam onward, is described as having "begot a son in his likeness, after his image." After the birth of this firstborn son, the men are described as having lived so many years and begot sons and daughters. The text then turns to that firstborn son, and in turn to his firstborn son after him. Women appear as the "daughters of men who bear them offspring." In a patriarchal kinship system, children are reckoned as being born to men, out of women. Women, in this system, bear the children of men.

In some societies, in contrast, the line of descent is not from father to son, but along the lines of the women. These are called "matrilineal" societies: it is a shared mother that makes for shared lineage or family group. Men still rule in these groups, but they do not rule as fathers. They rule the women and children who are related to them through their mother's line. Women in such a system are not a vulnerability, but a source of connection: people are not men's children coming through the bodies of women, but the children of women.

In a patriarchal system, the essential concept is the "seed," the part of men that grows into their likeness within the bodies of women. Such a system inevitably is male-dominated, but it is a particular kind of male domination. Men control women as daughters, much as they control their sons, but they also control women as the mothers of men's children. It is women's motherhood that men must control to maintain patriarchy. In a patriarchy, what is valued is the relationship of a man to his sons. Women are a vulnerability that men have, because to beget these sons, men must pass their seed through the body of a woman.

In a patriarchal system, when people talk about blood ties, they are talking about a genetic tie, a connection by seed. In a mother-based system, the blood tie is the mingled blood of mothers and their children: children grow out of the blood of their mothers, out of their bodies and being. The shared bond of kinship comes through mothers. The maternal tie is based on the growing of children. The patriarchal tie is based on genetics, the act of impregnating.

Each of these ways of thinking leads to different ideas about what a person is. In a mother-based system, a person is what mothers grow: people are made of the care and nurturance that bring a baby forth into the world and turn the baby into a member of society. In a patriarchal system, a person is what grows out of men's seed. The essence of the person, what the person really is, is there in the seed when it is planted in the mother. Early scientists in Western society were committed so deeply to the patriarchal concept that it influenced what they saw. One of the first uses of the microscope was to look at semen and see the complete little person, the homunculus, curled up inside the sperm (2).

Out of the patriarchal focus on the seed as the source of being, on the male production of children from men's seed, has grown our current thinking about procreation. One direction in which this has taken us is the focus on genetic determinism, whether in its most horrifying form, eugenics under the third Reich, or in its contemporary forms. In the United States, in the face of enormous and obvious social problems that cause disease and illness, the search for genetic causes of disease and illness has captured support. The United States has launched the human genome project, a phenomenal effort to track the genetic bases of human life and human illness. Thus we see that this belief in genetic determinism, the seed as the cause of our being, continues to flourish.

Modern technologies of procreation and modern genetics have been forced to go beyond the sperm as seed. Modern science has had to confront the egg as seed also. But that does not mean that we no longer continue to think of the seed as the essence of being. It is not the end of the belief that the seeds, the genes are everything, that they are what really matters in the making of a baby, the unfolding of a person, that they are what real kinship is based on.

The old patriarchal kinship system had a clear place for women, the nurturers of men's seeds, the soil in which seeds grew, the daughters who bore men offspring. When forced to acknowledge that a woman's genetic contribution was equal to a man's, Western patriarchy was in trouble. But the central concept of patriarchy, the importance of the seed, was retained by extending the concept to women. Valuing the seed of women, the genetic material that women also have, extends some of the privileges of patriarchy to women. Specifically, this has given women some rights to their children, rights based not on their nurturance and care of these children from conception onward, but rights based on their genetic connection.

This does not end patriarchy, and it does not end the domination by men of the children of women. Instead, by maintaining the centrality of the seed, the ideology maintains the rights of men in their children, even as it recognizes something approaching equal rights of women in their children. In the United States, men's control over women and the children of women is no longer based simply on men's (no longer) unique seed. Men's economic superiority and the other privileges of a male-dominated social system have become increasingly important. Children are, based on the seed, in American law "half his, half hers," and might as well have grown in the backyard. American men win custody battles for children not because they are entitled to the children based on the genetic connection, but because they are better equipped, based on economic advantages.

This is the context in which the elaborate new technologies of procreation are understood. For example, we have a technology that takes Susan's egg and puts it in Mary's body. And so we ask, who is the mother? Who is the surrogate? Is Mary substituting for Susan's body, growing Susan's baby for Susan? Or is Susan's egg substituting for Mary's, growing into Mary's baby in Mary's body? In the United States, we have used the technology both ways. When Mary's husband wants a child, he and Mary can purchase Susan's egg, use Mary's husband's sperm and Mary's body, and grow their "own" baby. But when Susan's husband wants a child, he and Susan can rent space in Mary's body, use Mary as a vessel for their baby. In the United States people with money can contract for pregnancy. The brokers have books of pictures of women for potential parents to choose from, to take this woman or that woman to carry the pregnancy, to nurture the seed.

Just as upper class men and women historically have been able to use the milk of poor women to raise their children, upper class men and women are now using the bodies of these poor women. Upper class women can have some of the privileges of patriarchy, using the bodies of poorer women to "bear them offspring." And upper class women can, as they so often have, be bought off with these privileges, and accept men's world view as their own. And so in the United States, we have women, right along with men, saying that what makes a child one's own is the seed, the genetic tie.

The ideology of patriarchy thus shapes women's as well as men's view of motherhood. Physical motherhood, the nurturance and intimacy of pregnancy, is made meaningless, whereas paternity, the genetic connection, becomes all-meaning.

The Ideology of Technology

In a technological society, we apply ideas about machines to the physical and social world. In the Netherlands, for example, the outsider is struck by the passion for control and order of the land. Never in my life have I seen a countryside, a landscape, with so many straight lines, so many right angles. No creek meanders, no path twists. Seas are placed deliberately in awe-inspiring displays of rational control. American agribusiness moves us in the same direction. Technological ideology asks people, like the land, to be more efficient, productive, rational, and controlled. A technological society treats our bodies as machines, hooking them up to other machines, monitoring and managing bodily functions. When a doctor manages a woman's labor, controlling her body with drugs and surgery, it is to make her labor more efficient, predictable, rational. And so it is when mothers and fathers push their babies onto a schedule, so that feeding the baby meshes with the workday. Books like "Toilet Training in One Day" have become bestsellers in the United States, showing us how to "train" our children efficiently. When we think of parenting, or raising our children, of our relationships with our children as a job to be done well, than we have invoked the ideology of technology.

The most obvious application of technological ideology to American motherhood has occurred in the medicalization of pregnancy and of childbirth. From the medical management of pregnancy with the new quality control technology of prenatal diagnosis, through the rigidly monitored and controlled labor typical of an American hospital birth, the focus is on the "mechanics" of production, and not the social transformation of motherhood.

In the American management of childbirth we see the ideology of technology played out in all its inhumane absurdity. Pregnant women are seen as workers in an unskilled assembly line, conceptualized as machines, containers holding precious genetic material. One or more ultrasound sessions have become fairly standard practice in American obstetric care. It is no longer quickening that marks the first realization of the baby as a separate being within, but rather the moment it is first seen on the screen.

Women report that in that moment of seeing the fetus on the ultrasound screen, it suddenly becomes real to them: real is probably the most often used word to describe the fetus on the screen. It is not that surprising that the first generation of women grow up watching television now feel their first real connection with their pregnancies and their coming babies when they seem them on a television monitor. Like starvation in Somalia or the earthrise from the moon, television makes the unimaginable real for people. The screen visually disconnects the woman from the fetus. The woman herself and her doctor both look away from the belly to the screen to see the baby. The feeling of direct contact with the fetus that ultrasound gives the physician changes the relationship between the woman and the physician, between the fetus and the physician. American obstetricians now routinely speak of the fetus as their patient, and of the mother as the "maternal environment," or even "maternal barrier."

The conceptual separation of mother and fetus continues in the management of birth (3). Virtually all American women go into the hospital, where the medical, obstetrical, and mechanical reality overrides the human drama of birth. In almost a parody of itself, the management of a birth in American means hooking the laboring woman up to a machine, a "fetal monitor." Fetal heart tones can even be read from a central location, with less and less need for direct contact with the laboring woman.

American obstetricians are entranced with idea of "managing" labor. The earlier focus was on the control of pain in labor; more interest is now aroused by controlling the length of labor. Studies report the wondrous advantages of aggressive management, the use of drugs to keep contractions coming every 2 minutes, to have the woman birth the baby within 12 hours or do a cesarean section. None of the studies even ask the women how they feel about any of this. For most American women, the physical experience of pregnancy and birth thus becomes transmuted from an expression of power, to one of dependence, as the woman experiences her body as a recalcitrant machine.

In a technological ideology, it is not only the body that is treated as a machine, as mechanical, but everything from the physical world around us to the social order. Carolyn Merchant, in *The Death of Nature,* traces the development of a mechanical order in Western society to replace the organicism of earlier times. Whereas in the time of the Renaissance, the earth was perceived as alive and considered to be a beneficent, receptive, nurturing female, by the end of the seventeenth century, the world view had changed and the machine became a metaphor. Merchant (4) writes, "Nature, society, and the human body came to be seen as composed of interchangeable atomized parts that can be repaired or replaced from the outside." This, she says, is the ideology that has permeated our consciousness. You can move a sea, transplant a liver, contract a pregnancy.

With this shift in the vision of the body and the land came a shift in the image of human relationships: "A new concept of the self as a rational master of the passions housed in a machine-like body began to replace the concept of the self as an integral part of a close-knit harmony of organic parts united to the cosmos and society." (4) Liberal philosophy, the intellectual underpinning of the American revolution and of American government, is the articulation of this technological ideology in the social order. When the authors of the American Constitution declared "All men are created equal," they were drawing on this philosophical insight. The equality they spoke of was of the mind, the rational being. Certainly some were weak and some strong, some rich and some poor, but all shared the human essence—the rational mind. The extension of such equality to people of color and to women is based on the claim that these groups too share the essence of humanity, the rational mind, housed by the "accident of birth" in the body of the woman.

If we believe, as this liberal philosophic tradition holds, that what is especially valuable about human beings is the capacity for rationality, then the ordering, rationalizing, and purposeful efficiency of technology will be seen as a good. But hand-in-hand with the valuing of rationality goes a theoretical disdain for significance of the body, and a disdain for physical work in preference for mental work. The latter, dividing the physical from the mental work, and then using machines and people interchangeably to do the menial physical work, is the essence of technological organization.

This division of labor is a particular problem for women as mothers; mothers do the physical work of the body, the menial work of body maintenance. Thus, women become identified with the physical, the body, and men with the higher, the rational. This distinction goes a long way toward explaining the utter disdain with which an American woman is strapped down, ignored, or even insulted, while the doctor who "manages" her labor—reading her chart and ordering others to carry out his decisions—is held in such high esteem. Or similarly, why the woman who produces perfect nourishment from her body for her baby is seen as "cow-like" while the pediatrician who "prescribes" a formula receives such respect—and high pay.

If it is the mind that is valued so clearly, and the bodily work so clearly disdained, then using one's body as a resource makes sense. The liberal tradition can thus support prostitution, so long as it is done on the basis of ostensibly free choice. The notion of "surrogate motherhood" is an even better example; the American liberal focus turns to the issue of informed consent, legal representation, and rational and deliberative choice. If the woman freely chooses to use her body as a rentable commodity, the liberal tradition in American law would uphold any surrogacy contract into which she may enter. The baby, the product of seed, is merely planted within her body, and she is making rational use of her body.

In sum, the ideology of technology, as an underpinning of the authoritative knowledge medicine claims, has order, predictability, rationality, and control as its basic themes. It brings with it the rationalization of life, the systematizing and control of things and of people as things, the reduction of all to component parts, and ultimately the vision of everything, including our very selves, as resources.

The Ideology of Capitalism

I will be speaking of capitalism in two ways. First, as an ideology like technology and patriarchy, which explains the practices and the contradictions surrounding American motherhood. Later I also will discuss capitalism as a practice, as a mechanism, as the way that American motherhood and American ideas about motherhood are marketed throughout the world.

Capitalism, like other ideologies, is complex, but I am forced to be brief, and inevitably must simplify it. Here I will focus on only one essential aspect of the capitalist ideology's contribution to the meaning of motherhood, the idea of ownership of the body.

Capitalism as an ideology, as well as a system, encourages the extension of ownership or property relations in ways that are inappropriate at best, and too often morally wrong. There are a great many contemporary social critics who make exactly that claim, including, for example, the ecologists. They argue that it is inappropriate to think we can own the land, the waters; they claim significantly that the earth is our mother, not our property.

But what happens when we start thinking of motherhood itself in terms of property? As wrong as it is to think of the human relationship to the earth this way, it is worse when we start thinking about our relationships with each other in the language of property. People do not often use the actual word "property" in discussions of human relations. The key term more often is privacy, with the concept of property being implicit. There are two directions in which property rights have extended that are directly relevant to motherhood: rights of ownership over one's children, and rights of ownership over one's body. It is the latter on which I will now focus.

In American society as a capitalist system, one cannot legally force or forcibly prevent people from doing something with their own property without very compelling reasons. The right to own and therefore to control property is among the most valued of rights. In a technological society, I have argued, the body is treated as a thing and as a resource. In a capitalist society, where the emphasis is on private ownership, the body is viewed not as a resource for the community or the society, but as private property, a personal resource. Rights of privacy are, in a sense, just a variation on other rights of ownership, of private control.

Given the view of the body as owned property, the extension of ownership to all of their body parts allows women some measure of control over the use of their bodies in procreation. If women are full persons, then they are moved out of the category of owned property, and into the category of owners of their own bodies. Men can no longer (legally) barter in women's bodies.

American feminists have made good use of this concept of bodily ownership. Within the American legal system, intelligent feminist use of the individualist ethos has been invaluable in assuring women's rights in procreation. Once women themselves are recognized as full citizens, then individual women must be accorded the same rights of bodily autonomy and integrity that men have. For women, that has meant sexual and procreative autonomy. Because it is her body, she cannot be raped. Because it is her body, she cannot be forced to bear pregnancies she does not want. Because it is her body, she cannot be forced to abort pregnancies she does want.

This does not mean that American women are not forced by circumstances into these very situations and eventualities. It only means that the society will not use the official power of the state to force them. Women in fact are prevented from having abortions they might want by family pressure, by economic circumstances, and by religious and social pressures. And women in America are forced into having abortions they might not want to have because of poverty, because of lack of services for children and mothers, because of lack of services for disabled children and adults. By offering amniocentesis to identify fetuses who would have disabilities, and by cutting back on services for disabled children and their families, for example, we effectively force women to have selective abortions.

But we have held the line on adding to that pressure the official power of the state. In American society, when we return to the simple legal questions—who can force an abortion or forcibly prevent one, force a woman to have sex or stop her from doing do, to use contraception or not—we wisely retreat to safety, calling forth our most sacred value. It's *her* body. We invoke a higher power, the power of ownership.

This then is the way that American women have been able to combine the dominant American liberal philosophy with capitalist ideology to our benefit. We have made use of the mind-body dualism, to allow a view of the body as owned, like a shelter which houses the more important mind. The high value of ownership, of property rights, is then turned to the advantage of women who can claim exclusive property rights to their own bodies. In the name of ownership, American women have demanded access to contraception, sterilization, and abortion. And given the prevailing liberal philosophy, they have obtained rights to control their fertility—although given the capitalist class system, they have fared less well with access to the necessary means.

Although the "owned body" principle has worked for women in avoiding motherhood, it is less clear how it can be made to work to empower women as mothers. Our bodies may be ours, but given the ideology of patriarchy, the bodies of mothers are not highly valued. The bodies are merely the space in which genetic material matures into babies. In a patriarchal system, even if women own their bodies, that ownership may not give them any real control in pregnancy. Women simply may be seen as owning the space in which the fetuses are housed. This is the argument on which attempts to control women's behavior has been based successfully; owning her own body is not enough to assure a woman her civil liberties if her body is believed to contain the property of someone else, someone else's baby. Thus American women literally have been jailed for using drugs in pregnancy, literally been dragged against their will into operating rooms for cesarean sections.

Weaving the Fabric

From the standpoint of the ideology of technology, we have seen that motherhood is perceived as work, and children as a product produced by the labor of mothering. Mothers' work and mothers' bodies are resources out of which babies are made. From the standpoint of the ideology of patriarchy, it is men's babies that are being made. From the standpoint of the market, not all work is equally valuable, and not all products are valued equally. There is not a direct relationship between the value of the worker and the value of the product. What is essential to capitalism is the accumulation and investment of capital, of wealth, by people who are in a position to control others. Under capitalism, workers do not own or control the products of their own labor.

Babies, at least healthy white babies, are very precious products in the United States these days. Mothers are the expendable, cheap, not-too-trustworthy labor necessary to produce the precious product. This is the end result of the evolution of these three ideological perspectives. This is what ties together the new technology and the old technology, the legal, the medical, the political, and the psychological creation of motherhood in American society. From these three ideologies, those of patriarchy, technology, and capitalism, we get the supportive fabric for the strange patterns we see emerging in the United States, patterns which may already begin to threaten other countries. In combinations, sometimes with one thread dominating and sometimes another, these three ideologies explain the contradictions with which I began. These ideologies also explain the direction toward which the developments of the new technologies of procreation are taking us: further and further toward treating babies as commodities to be produced out of precious genetic material, mothers as "cheap labor," untrustworthy workers on a reproductive assembly line.

It is the nature of a hegemony—this complex world view constructed of interlocking ideologies— that we cannot see through it clearly. And so we fear to pick at the fabric, fear to pull at individual loss threads for fear of falling straight through into some abyss. If we challenge any piece of the system, other pieces rise to block our way. When I challenge technological ideology, people hear the sound of the baby being chucked out with the bathwâter, fear the return of the angel of death hovering over every birth, fear unchecked fertility, untreatable infertility, women captured and held hostage to some mad biology. When I challenge ownership models of bodily integrity, I hear the enormous fear of someone else claiming ownership. The ownership model lies so deeply in American society that the only question to be asked is whose property. And when I challenge the patriarchal models of gene-based parenthood, I hear the fears of women of privilege who have gained for themselves some of the privileges of patriarchy, often at the expense of other women—often, in America, at the expense of women of color. If I talk about nurturance, daily acts of caring, of tending, of raising children as constituting motherhood, then women who hire other women to perform those acts in their absence, as babysitters, housekeepers, and nannies, feel their motherhood claims threatened.

Thus, we in the United States feel ourselves trapped in a tightly woven, apparently seamless fabric. We have come to think about motherhood and about our children in certain ways, and we have developed technologies out of that thinking. The technologies in turn act back on us, further encouraging our ways of thinking. We think of the body as a machine, and so develop machines to attach to the body until finally the body—the laboring body of a woman with child—appears to be an extension of the machinery. We think of the woman's body as a barrier to access to the child planted within, and develop technology that makes the woman invisible, opens up the fetus to our eye, makes it appear as the trapped child we thought it was. We think of seed as precious, and develop techniques for harvesting it, for culling it, for selecting the most precious seeds. We think of the site of growth of those seeds as interchangeable, and we interchange them. We create a world in our image.

And then we sell it.

Selling the Vision

It begins in a way that generally makes some sense, that pulls on your heart strings, that captures your imagination. We start with some woman in a very high risk situation: the technology offers her a chance, maybe her only chance, to have a baby. But then very quickly the technology begins to spread to people at lower and lower risk, until virtually every woman is caught up in the net. In the United States, the cycle may take just a few years. Often, once the technology in fact is routinized, the problems with it, the problems it causes rather than solves, are discovered, and the technology is abandoned. Usually it is abandoned in favor of some newer technology, and the cycle starts over.

Such was the story of x-ray use in pregnancy: from a few women with abnormal pelvic structure to routine use in the population, to discovery of the link to childhood cancers, to replacement with ultrasonography. It is the story of weight control programs for pregnant women, or prenatal diagnosis, of fetal monitors. Always it begins with some heart-rending crisis. Always it ends with the routinization of the technology.

I met a physician in the Netherlands at a conference on the use of prenatal diagnosis. I was critical of the introduction of the technology of prenatal diagnosis for routine use. Ah, she said, but don't you see: She had just come from the bedside of a woman who was recovering from her second elective abortion following prenatal diagnosis. She and her husband were carriers of a dreadful condition, something no one would wish on their own children. The decision to test for that condition in the fetus and to terminate the pregnancy if the condition were found, was their only hope of having a healthy child. Didn't I see how fine a thing these tests were, how valuable they could be?

They were carriers, I asked? For a child to be affected, the genes had to be present in both the sperm and the egg? Yes, yes, she assured me, that's what put them at such high risk. Did you offer them donor insemination, I asked? If they used donated sperm, they would have no risk of this condition. The physician was silent for a long moment. I never thought of it, she said, I just never thought of it. When she had left them earlier that day, the man was sitting at the bedside with his head bowed over his wife's empty belly, weeping. He might have accepted donor sperm, she thought.

The couple was using the technology of prenatal diagnosis in this case not simply to have a healthy baby (2). They were using this technology, this particular technology to have a healthy baby, in order to maintain the husband's paternity. And it was not even necessarily out of the husband's desire to do so—it was the doctor's decision to offer this technology. Donor insemination is an inexpensive, low-technology, low-risk, low-profit treatment. There is no international marketing program for donor insemination. The technology of prenatal diagnosis that the physician was offering is a technology that is being marketed aggressively throughout the world. It is a technology that grew out of the complex weave of ideologies I have been describing, but wherever these technologies originate, whatever ideology or way of life they grow out of, they are marketed throughout the world.

The conference at which I met this physician was sponsored partly by the pharmaceutical companies that make the prenatal diagnostic kits. The audience consisted of obstetricians who would be doing the testing. The specific test under discussion was to be used in low-risk pregnancies, possibly eventually in all pregnancies, to screen people for whom further testing would be advised. The doctor was showing me the path from the tragic couple at high risk to the whole population, the path by which acceptance of this technology could be routinized in this society.

The technology that is developed in any particular society is no longer simply a reflection of the cultural values of that country. It is also a product, an object subject to multinational development, international marketing. Once we have an object, a marketable commodity, the power of the multinational industries in the global economy begins to enter the picture. All the physical means of production of motherhood in the United States—fetal monitors, screening kits for the prenatal detection of fetuses with Down syndrome, ultrasound machines, infant formula, all the products used from the lowest technology baby bottle to the highest technology DNA probe—are subject to international marketing. Many of these objects have been developed in the United States; some are imports. The culture of science, the world of reproductive technology, is itself an international world, and products and people move around quickly.

The case of in vitro fertilization and the other infertility research is a good example. As one country after another, including the United States, imposed various restrictive bans on fetal and embryo research, the scientists and researchers moved their laboratories from country to country. The technologies they then developed, no longer considered "research" or "experimental," are now marketed throughout the world. The conferences are held internationally; the science develops its own international culture. But the marketing gets targeted, like all international marketing, addressed to the local market. IVF Australia opens up branch outlets wherever rich people with infertility problems can be found. In the United States, it calls itself IVF America. IVF clinics now blanket the world.

Some technologically inappropriate marketing across the globe costs lives: this has been the well-documented case with the marketing of infant formula to underdeveloped countries. As the mother of one child by adoption, I deeply value the availability of good infant formula. But clearly the inappropriate marketing of the formula for profit has cost lives. I am not arguing against these technologies. I am not opposed to all uses of IVF, or of prenatal diagnosis, any more than I am opposed to all cesarean sections. But I am very aware that these technologies are often not used in the best interests of women, that they grow out of an ideology that makes them dangerous. A needed cesarean section is a lifesaver; a 24% section rate is madness. To know where a technology is headed, it helps to know where it is coming from.

Choices in procreation, like all other choices in life, do not occur in a vacuum. They occur in a political, ideological, and economic context. The American focus on individual choice often blinds us to these larger questions. We find ourselves focusing on the narrow ethical concerns of fair treatment of the individual, assuring fully informed consent, unconstrained choice, psychological counseling. As if full information were ever available, as if choices were ever not constrained, and as if psychological counseling solves political and economic dilemmas.

Standing within the tightly woven fabric of her life and life-world, a woman does what she can, does what she has to. Certainly, information, choice, and support are needed. They are necessary, but they are not sufficient to address the concerns raised by technologies of procreation. It is not the woman alone, but the context that must be attended to. Many of us, feminists in the United States and throughout the world, are seeking a new tapestry, woven from the threads of different ideologies. We want to replace patriarchy with a woman-centered, woman-grounded vision that values nurturance, the work, and the care of mothering. We want to replace mechanistic, technological models with a valuing of the body, the organic oneness of the human body and mind, the interconnectedness of the human community. And we want to remove all images of ownership, of property, and of profit from the realm of mothers and children.

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Cases and Doubts

Randi E. Zinberg, M.S., M.S.

Case 1: Tay-Sachs Disease

Infantile Tay-Sachs Disease

Tay-Sachs disease (TSD) is a fatal genetic disorder in children that causes progressive destruction of the central nervous system. It is caused by the absence of the enzyme β-hexosaminidase A. Deficiency of this enzyme leads to the buildup in the cells of a lipid called GM2 ganglioside, particularly in the nerve cells of the brain. A child with TSD appears normal at birth, but evidence of the disease begins to appear by approximately 6 months of age. The affected child begins to lose developmental skills because of involvement of the nervous system. Loss of coordination, seizures, progressive inability to swallow and inefficient pulmonary function increase. Eventually, the child becomes blind, severely retarded, paralyzed, and unaware of the outside world. There is no treatment. Death inevitably occurs, usually between 3 and 5 years of age.

Tay-Sachs disease is inherited as an autosomal recessive disorder. This means that each parent must be a carrier for TSD, and that as a couple, with each pregnancy, they have a 25% risk of having a child with the disease. An individual's risk of being a Tay-Sachs carrier is greatest if he/she is of Ashkenazi Jewish descent. Approximately 1 in every 25 persons of Ashkenazi Jewish descent is a carrier of the Tay-Sachs gene. Tay-Sachs disease also can occur in Jews of Sephardic origin and in the non-Jewish community, although the frequency is much lower. Carrier screening for TSD by measuring the enzyme β-hexosaminidase A in peripheral blood has been available since the early 1970s. Couples determined to be at risk of having a child with Tay-Sachs disease have the option of prenatal diagnosis by either chorionic villus sampling or by amniocentesis.

In the past few years, DNA-based gene tests have been used as an adjunct to the enzyme measurements for carrier testing. Two common mutations causing the infantile form of TSD have been identified in the hexosaminidase α-chain gene.

Late Onset Tay-Sachs Disease

Late onset Tay-Sachs disease is a relatively rare form of Tay-Sachs disease. Onset of the disease occurs during childhood or adolescence and is characterized by incoordination, tremor, and/or slurred speech. With advancing age, patients develop neurologic symptoms including ataxia, unsteady gait, muscle weakness, and slurred speech. In the fourth decade of life, mental and behavioral involvement may become evident. Late Onset Tay-Sachs disease is inherited as an autosomal recessive disease. The mutation in the hexosaminidase A gene that causes this form of the disease also has been identified. An individual with one "infantile" mutation and one "adult" mutation will have this disease. It should be noted that routine enzymatic carrier screening does not distinguish between the common infantile form and the rarer late onset form of the disease.

Case 1

Mr. and Mrs. H. were 41 and 42 years old, respectively. They had no children. After years of infertility they pursued in vitro fertilization (IVF). The first attempt resulted in a miscarriage; the second, a twin gestation.

The couple sought genetic counseling when Mrs. H. was approximately 17 weeks pregnant. After an ultrasound examination, the couple

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Address correspondence to Randi E. Zinberg, M.S., M.S., Department of Human Genetics, Box 1497, The Mount Sinai School of Medicine, One East 100th Street, New York, NY 10029.

wanted to discuss amniocentesis in view of Mrs. H.'s age-related risks for chromosome abnormalities. Family and medical histories were unremarkable. They indicated that they were of Ashkenazi Jewish descent. Within the past few weeks, Mrs. H. had been screened for Tay-Sachs disease. Based on enzyme analysis, she was found to be a carrier. Mr. H.'s results were pending.

Enzyme analysis subsequently determined that Mr. H. also was a carrier. Therefore, statistically, each fetus had a 25% risk of being affected with this disease. The couple opted for screening the fetuses for Tay-Sachs disease.

To provide state-of-the-art prenatal testing, mutation analysis was performed on Mr. and Mrs. H. A common infantile mutation was detected in Mr. H., but the mutation identified in Mrs. H. was the adult mutation. Therefore, the couple was not at risk for the infantile form, which was what concerned them most. The results of the amniocentesis revealed that Twin A was affected with TSD, and Twin B unaffected. The couple had the following options: 1) selective termination of the affected fetus, 2) placing Twin A for adoption, or 3) no intervention.

The couple opted to continue the pregnancy with no intervention.

Discussion

Screening for Tay-Sachs disease in the Ashkenazi Jewish population has been the prototype of a successful screening program. More than 1100 carrier couples have been detected, allowing them to pursue prenatal testing and terminate an affected fetus. In the past, many couples who had a child with TSD did not risk attempting another pregnancy. Prenatal diagnosis allows couples determined to be at risk to achieve their desired family size without having to live through the tragedy of having a child with TSD, or a pregnancy filled with anxiety about TSD.

The case above illustrated some of the complexities surrounding genetic testing. Mr. and Mrs. H.'s decision was influenced by a variety of factors. Mrs. H. was approximately 20 weeks pregnant by the time they learned of the results. This pregnancy had been difficult to achieve and perhaps would be her last one. Selective termination, although a relatively safe procedure, is not risk free, so they opted against it. Moreover, they learned that the fetuses were not at risk for the fatal infantile form of the disease. Thus, they chose to continue the pregnancy and hoped that future therapies might be developed to mitigate the severity of the late onset form of the disease in the affected fraternal twin.

The entire circumstance could have been avoided if early counseling and screening had been carried out before the IVF. This would have allowed time for decision making and early prenatal testing, possibly even before implantation. This case makes it clear that IVF programs routinely should offer carrier screening to all patients before treatment.

Case 2: Gaucher Disease

Gaucher disease is one of the most common inherited disorders affecting those of Ashkenazi Jewish descent. During the past few years, dramatic advances have been made in dealing with this disease, including gene-based diagnosis, carrier detection, and the development of effective enzyme replacement therapy.

There are three clinical subtypes of Gaucher disease which are distinguished by the absence or presence and severity of neurologic complications. Type 1 disease, which does not involve the nervous system, occurs with high prevalence among Ashkenazi Jews. About 1 in every 19 Ashkenazi Jewish individuals is a carrier of the gene that causes Gaucher disease and approximately 1 in 1500 are affected. Type 2 disease, which is very rare, is a fatal neurodegenerative disorder of infancy, similar to Tay-Sachs disease. Type 3 disease is a slowly progressive neurologic disease with survival into adulthood. The occurrence of Types 2 or 3 Gaucher disease among Ashkenazi Jews is rare.

Gaucher disease results from the deficiency of the enzyme acid β-glucosidase and the subsequent accumulation of an unmetabolized substance called glucosylceramide. This substance is deposited in unique cells, referred to as Gaucher cells, in the spleen, liver, and bone marrow. Accumulation of Gaucher cells leads to enlargement of the spleen and liver as well as blood abnormalities including anemia and impaired blood clotting with easy bruising. Gaucher cells in the bone marrow may lead to bone and joint pain or fractures. The clinical manifestations of Type 1 Gaucher disease usually become apparent in childhood or early adulthood, when patients initially develop an enlarged spleen, anemia, low platelet count, or orthopedic problems. The recent development of specific therapy by enzyme replacement has provided a form of treatment. To date, this approach has proven safe and efficacious in treating many of the major manifestations of Type 1 Gaucher disease.

The gene for acid β-glucosidase has been isolated and common mutations in the gene which causes Type 1 Gaucher disease have been identified. Screening for these mutations can detect 90 to 95% of carriers. Carrier screening for these mutations is more accurate than screening for this disease by measuring the enzyme. However, an individual can be determined to have Gaucher disease by measuring the enzyme in peripheral blood and by looking for specific mutations. At risk couples have the option of obtaining prenatal diagnosis by chorionic villus sampling or amniocentesis.

Because Type 1 Gaucher disease is a variable disorder, it is difficult to predict the severity and extent of complications in individual patients. Some patients may be affected severely in childhood or teenage years, whereas others are relatively asymptomatic through their 50s or 60s. Although there is no classic predictable disease course, prognosis generally depends on the severity at diagnosis and the occurrence interval between incidents of disease complications in each patient. The possibility of trying to predict disease severity by the specific mutation(s) identified (genotype/phenotype correlations) is under investigation. Individuals who are homozygous for the common N370S mutation may tend to have the less severe form of the disease, with onset later in life, whereas other mutations or N370S combined with other mutations may lead to an earlier and more aggressive form of the disease.

Case 2

Mrs. G. was pregnant and both she and her husband strongly believed in carrier screening to obtain as much information as they could. After discussing the screening tests available for various disorders, i.e., Tay-Sachs disease, cystic fibrosis, Canavan disease, Niemann-Pick disease, and Gaucher disease, they opted for screening for all five. Results determined that Mr. and Mrs. G. were both carriers for the N370S mutation in Gaucher disease and therefore had a 25% risk of having an affected child. They pursued amniocentesis for prenatal diagnosis. The fetus was determined to be affected. After extensive counseling, they made the decision to continue the pregnancy based on what was known about the disease, the mutations involved, and the availability of enzyme replacement therapy. Mr. and Mrs. G. expressed the view that their decision might have been different if the mutations they carried had indicated the possibility of a more severe disease course.

Comments

Carrier screening for genetic diseases which often do not display symptoms until adulthood will increase. Many health professionals are concerned that the knowledge, before birth, of a condition that may not manifest itself for decades will cause harm, for example, will interfere with parent/child bonding. On the other hand, others argue that the knowledge may not interfere with the normal bonding process and that the knowledge could allow for early medical intervention and the initiation of appropriate treatment/therapies which maximize quality of life. These complex issues have to be studied to determine the extent of the potential for harm. With the results from these studies, new counseling strategies can be designed and implemented to assist parental decision-making.

How Perfect? A Case of Prenatal Diagnosis

Barbra Paula Bluestone, M.D.

The Case

A 21-YEAR-OLD WOMAN who was pregnant was followed up in the gynecology clinic. At 18 weeks of gestation, the patient was scheduled to have the maternal serum α-fetoprotein test done and a routine ultrasound for dating. The biparietal diameter and abdominal circumference of the fetus were noted to be consistent with 18 weeks but the long bones were found to be consistent with 15 weeks. The patient was scheduled for an appointment with a genetic counselor the next day.

At 22 weeks of gestation, the patient was informed that her amniocentesis results, including α-fetoprotein, were normal. Her fetus was a female with a normal number of chromosomes and with a normal banding pattern. Markers for achondroplasia were absent. She was informed, at this point, that all available tests other than the ultrasound revealed a normal fetus but certain skeletal dysplasias could not be ruled out. The patient decided to continue the pregnancy.

An ultrasound 3 weeks later revealed long bones 4 weeks behind gestational age that subjectively appeared somewhat bowed. Follow-up ultrasound at 27 weeks measured a normal femur but a humerus 2 weeks behind gestational age. Subsequently, ultrasound examination revealed short but growing long bones which was reassuring to the maternal-fetal specialists.

At 41 plus weeks, the patient was delivered of a healthy baby girl, 6 pounds, appearing normal in all respects including limb size.

Discussion

The unhappy possibilities and anxieties generated by imperfect technology finally were resolved with a happy ending. Our patient was given the most information available but it ended up being misleading despite all our best efforts. Sometimes too much information can be as misleading as too little.

Screening tests for congenital anomalies should be designed to pick up the 3% incidence of major anomalies that accounts for 20% to 30% of perinatal deaths (1). Consider ultrasound as a screening test for fetal abnormalities. The recommendations from experts is confusing and remains controversial (2). In 1984, a consensus statement from the National Institutes of Health recommended against the use of routine ultrasound whereas the Federal Republic of Germany passed a mandate requiring a routine sonogram in all pregnancies (3, 4). At best, the sensitivity for picking up a fetal abnormality is 80% and this number varies considerably depending on the organ system involved. In addition, maternal obesity, multiple pregnancy, and fetal position may be barriers to a routine ultrasound. To assure a pregnant woman that everything looks "just fine" after what appears to be a normal ultrasound is misleading. In some studies, the sensitivity of picking up abnormalities includes abnormalities of unknown origin or of no significance, as for example, a dilated renal pelvis or choroid plexus cyst. Abnormalities such as limb length are based on averages of normal fetuses and then linked to body proportion, so that a femur length of 22 weeks should correspond with biparietal diameter and abdominal circumference of 22 weeks if the fetus is proportional on average. In any given situation, the data may be outside of the range

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Address correspondence to Barbra Paula Bluestone, M.D., Department of Medicine, Mount Sinai School of Medicine, New York, NY 10029.

Key words: Prenatal diagnosis

found in the usual normal distribution. Nevertheless, if this baby had been born with a skeletal dysplasia, no one would have been very surprised. In 1994, the much anticipated RADIUS study concluded that ultrasonography screening had no place in a low-risk population, but a storm of criticism followed the study (5, 6). Most women in whom an abnormal fetus was diagnosed terminated their pregnancies and there is no mention as to whether or not the aborted fetuses were examined for the diagnosed abnormality. Our case reflects less-than-perfect specificity.

Patients demand ultrasound despite its equivocal sensitivity and specificity because of its alluring pictorial aspect. We believe what we see; thus a movie of our baby moving around appearing to be "just fine" is much more reassuring than a test value on a page. But the information that ultrasound has to offer raises some very important issues of patient autonomy and informed consent. In the ideal situation, a patient is informed of all available information and the relative significance and reliability of that information. The patient is then able to take her understanding of the information and apply it to the decision she needs to make. But with the controversy surrounding routine ultrasound in a low-risk population, how can a physician adequately explain the risks and benefits? Obviously, before any ultrasound, the physician and patient should discuss the possibility of an anomaly that may not be confirmed and the patient's feelings regarding termination. We do not know just how far this ideal communication is from the actual situation.

Prenatal diagnosis is designed to describe and predict the health of the future child. Fetuses terminated after prenatal diagnosis were wanted but wanted only under certain conditions, those of a certain physical and mental constitution. In that framework, what does the diagnosis of "just fine" or a "normal" fetus in prenatal testing mean? Does it mean 10 fingers and 10 toes, does it mean beauty queen, does it mean free from disease, free from future suffering? What should our goal be in our communication with patients?

Prenatal diagnosis is a rapidly developing technology. Guidelines regarding specific situations today are sure to be obsolete in the near future. Because we live in a society that acknowledges the importance of autonomy, we should be reluctant to regulate the decisions that women can make in light of information from prenatal diagnosis. It is inevitable in a pluralistic society that emphasis is on the rights of the individual rather than some kind of overriding idealistic aim of society. Love, tolerance, and acceptance within the home cannot be taught and enforced by a law-making body. This is the province of religious, philosophical, and educational leaders. Prescreening for genetic disease in at-risk parents is a good way to prepare people for their options in future childbearing. Excellent prenatal care with emphasis on those behaviors that prevent major anomalies, such as increasing folic acid intake and cessation of alcohol are clearly ethically uncomplicated ways to accomplish the goal of bearing fewer affected children. We need to continue to learn from surveys of the prenatal caregivers what their patients are saying they want from prenatal testing, and how well they are able to process information regarding their prenatal screening to make decisions with which they can be happy. How can we help those caregivers? We need to decide what the most important information is and disseminate it to communities to make prenatal diagnosis and the childbearing process safer, more efficacious and fulfilling.

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Panel Discussion

Issues in Medical Ethics

Rosamond Rhodes, Ph.D., and Daniel A. Moros, M.D., Moderators

Rosamond Rhodes: Let's start with a discussion of the cases presented. Do any of our panelists have comments?

Judith Willner: I hope physicians don't give patients the notion that we can guarantee perfect babies for them because that's not true. We need to inform our patients that there are risks for everybody. It is necessary to know how to give parents bad news, so communication skills should be part of physician training. Unfortunately sometimes we are put on the spot trying to figure out how to give parents bad news in as gentle and kind a way as we can.

Hirschhorn: One is caught in a funny situation when women are about to get pregnant or come in early in their pregnancy. Those who see a lot of abnormal kids are aware that somewhere around 3% of all pregnancies are affected by a major defect. The problem is, how do you tell this to prospective parents and what do you do about it? There are optimists and pessimists. The optimistic parents focus on the fact that there is a 97% chance of having a normal baby and so they are not worried. However, the pessimists note that there's a 3% chance of having an abnormal baby, and they want very much to find out how to avoid such an outcome. We can find out about some of the abnormalities. For example, we can find out whether there are chromosomal abnormalities, a few inborn errors for which they may be at risk. Some things are obvious on ultrasound, but there are lots of things we can miss. The question is whether information about such screening options should be part of how we formally educate every couple.

Barbra Bluestone: I think we need to have more communication with our patients. I had a lot of respect for the patient whose case I presented in choosing to carry through with her pregnancy. What is considered abnormal worries me. What would Down syndrome children who are living happy fulfilling lives think if they learn we are terminating pregnancies based on that diagnosis? It sends a message that maybe their lives aren't worth living. Attitudes toward disability cannot be mandated by law but they are something that society has to change.

Rhodes: Prof. Smith, given these cases, could you comment on certainty and predictability?

Kelly Smith: We have to keep in mind that it is sometimes difficult for us to recognize how hard this information is for the average patient to interpret. Physicians and counselors are familiar with this problem. A physician or counselor sits down with a patient and says, "You realize of course there is this risk," and then goes through some detailed description of the statistics. When I ask people what they got from the conversation the typical response I get is, "They said something really complicated and I have to have this test and when the test comes back they'll figure out what they are going to do next." Now I don't have a magic bullet to solve this problem, but I think it is a fundamental difficulty. Patients often don't understand what their doctor is saying, and often perceive any information being given as good information, otherwise why would the physician be talking about it in the first place?

Barbara Katz Rothman: You sound a lot like the midwives whom I interviewed in the Netherlands. It starts with the ultrasound (which is not routine in the Netherlands because it is believed that it doesn't work that well for anything), and it's just like opening up a sewer. Often you have a baby that's going to die or be whatever it's going to be no matter what you do and all you are going to do is spoil the pregnancy, making the woman miserable for a very long time first. Often everything is going to be all right, but you torture that poor woman for months making her imagine all sorts of terrible things—like the short woman

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Address correspondence to Rosamond Rhodes, Ph.D., Association Professor of Medical Education, Director of Bioethics Education, Mount Sinai School of Medicine, One East 100th Street, New York, NY 10029.

who is told that the fetus she is carrying has short limbs, which causes her months of anxiety from which she makes herself crazy, perhaps for nothing. What you've done, particularly with the very dramatic problem of the false negatives is, for example, you tell a woman she should be tested for Down syndrome. She says, "Yes, that's a terrible thing, so I'll have my α-fetal protein screening because a child should never be brought into the world with Down syndrome." She has the testing. Then, with the α-fetal protein screening the condition is missed and she ends up having a child with precisely that condition. Now she's raising that child after a careful, soul-searching decision that such a life is not worth living. In no way have you benefited or empowered that woman in the raising of that child. So, it's not simply a matter of making sure we explain it better to these patients, who are a little confused and nervous. One of my favorite expressions is that motherhood is, among other things, one more chance for a speeding truck to ruin your life. The notion that somehow or other you can test for it or assure against it or fix it is a troubling ideology. What is so unfortunate is that it directs the technology.

Hirschhorn: I think you are stating opinion as fact. Just because one women had troubles after the Down test for her child was falsely negative does not say anything about others being benefited tremendously. Other women who were involved in the same screening process could have been helped by the information.

Katz Rothman: The triple test doesn't have a 99% accuracy rate.

Hirschhorn: No but if it's positive, or apparently positive, you will do an amniocentesis and find out.

Katz Rothman: Yes, and if it's apparently negative, you don't, and the woman is going to end up with the kid anyway. I just ask you to bear in mind that the accuracy rate is not usually as high as 99%.

Rhodes: Knowing risks usually affects our behavior and so it's important to know these risks. So why is obtaining knowledge about genetic risks so different?

Katz Rothman: It's not particularly, and I'm not making the claim that it is. However, my point is that it is not just one doctor's bad communication to a patient that results in that patient expecting perfection. It's the societal concern with control in any and all areas of life, when life doesn't actually come with that much control. It's not just a matter of a miscommunication in a doctor-patient relationship. We are dealing with something that is deep within the ideology of the culture.

Richard Berkowitz: I don't really understand your message. Are you suggesting that we not look for anything? That we just take what we get? Is it that whatever has been predetermined to be the lot of the fetus is something that should just be accepted?

Katz Rothman: I didn't say that nor am I suggesting it. I was responding to the idea that the woman who has had a certain amount of screening expects perfection. Then she is shocked by the absence of it. It's the nature of the screens—they have both false positive and false negative rates. That's why they're screens, otherwise they'd be diagnostic tests. So, we end up with many people who have a lot of trouble as a result of this technology. That doesn't mean it should not be used or that it should be used twice as much. But we should stop treating each woman who ends up on the bad side of this as some interesting aberrant 1 in a 100, compared with the 99% who benefit. We're producing as much harm to them as we are benefit to the woman who gets the news that she wants or needed.

Berkowitz: That still doesn't answer my question. Should we have no screening? We don't live in a society where people think it's fine to have children with mental retardation. If we did, then I think it would be a very different message. We live in a society where people do make judgments about the kind of children they would like to rear.

Katz Rothman: I think the logical progression about to come is something like this: if you can clone from adults, we have probably 100,000 perfectly good models to choose from. Those who have lived to 95 with no obvious genetic diseases ought to be used and nobody should take all the risks of this genetic crapshoot. If we want to be rational, why should we take more risks? We are beginning to approach a nearly risk-free genetic technology where we can make some sensible rational choices, but it raises some other problems. The path of least risk is not always the best path to follow. I'm not making the claim that we shouldn't do the testing. I'm not making the claim that we should do the testing. I do think that we vastly oversimplify the problems and treat the problems in genetic testing as a miscommunication or overexpectation or occasional test error. I'm suggesting that the problems are built in deeply and if you are going to have the solution, you are also going to have the problem.

Patricia Mann: This particular dialogue exemplifies what I was trying to think out for myself. There are two very different levels of concern, or whole different worlds within which we're operating. There's the medical profession delivering phenomenal service as best it can, doing amazing things by which we can't help but be impressed, making people happy by positive, almost miraculous interventions. On the other hand, what Prof. Katz Rothman and I are talking about comes from a perspective different from that of the medical profession. In fact there are social values at work and there is a connection between social values and these powerful new technologies. Questionable values give exaggerated meaning to problems in some cases. Prof. Katz Rothman's emphasis is on one set of values, in particular about who is valuable and who is not. My emphasis is on the issue of the value placed on becoming a mother. I think, very reasonably, all of you have pride and confidence in the fact that you are helping each person and each family to have a family. I am not questioning that. I am questioning the larger cultural value placed on having children in spite of the contraindications given overpopulation, pollution, etc. From a larger perspective, traditional families do not exist so much any more. So the effort to reproduce them seems forced. However, individually, on the micro level, which is where in fact you are operating, they remain important.

Randi Zinberg: I was thinking that this discussion seems to be putting everyone in camps. There is the social view as opposed to the medical view. In fact, many of us think through these issues on many different levels. We think through them as individuals, as patients ourselves, and as providers of services in the social context. I think we need to recognize that many of the issues we raise are issues that we discuss and try to promote and it is in this way that we try to bring some sensitivity to our patients. Do we think this is difficult and overwhelming for the patients? Yes. Does it mean that many patients do not consider this a benefit to them? No.

Audience: If a family is given the diagnosis that they are going to have a child with Down syndrome would you explain the range of early intervention services available?

Willner: Yes, when patients have tremendous ambivalence, we offer them the opportunity to speak to patients who elected to continue their pregnancies.

Audience: Only if they exhibit ambivalence? If they say they want to terminate, is that it? Do you stop the discussion? Do you automatically give the entire range of options in truly value-free counseling? Which is to say, do you offer the entire range including perhaps meeting families who have kids with Down syndrome?

Zinberg: Yes, we describe the support groups that exist and give them the opportunity to contact those support groups. However, once a couple makes their decision, we don't say to them, "You cannot have a termination because you didn't call up and talk to anyone."

Hirschhorn: I think it's important to discuss the nature of genetic counseling. Genetics counseling is a communication process and it includes everything just mentioned. But the goal of genetic counseling—the end point—is for the family to understand their options, the outcomes. Once they do, they make their decision. The role of the counselor is to support that decision not to argue with them.

Katz Rothman: There are two problems. One is that bad counseling occurs. I have heard genetics counselors say to patients about children with Down syndrome, "They are cute children but they grow up to be ugly adults." So there are bad counselors, but there are always some people who are bad, regardless of the type of job. There are bad plumbers, bad architects, etc. Some people in every field do bad work. What's troubling to me is the narrowness of the focus. Women do not have full autonomy simply because they are in rooms with genetics counselors. They must make their decisions in a political and economic context, just like Sophie in *Sophie's Choice*. To say that a woman has autonomy in her decision to have a child who will need a wheel chair when she has been told about cuts in social services or knows she can only afford to live in her fourth floor walk-up apartment doesn't address the context in which her decision is being made. Knowing the down side before going in doesn't guarantee freedom of choice. Such contextual analysis can't be done by a genetics counselor. Counseling a women is not just a communication issue. I have met women who have terminated pregnancies when faced with a diagnosis of Down syndrome, women who spent years as teachers of children with Down syndrome. Although they may feel very positively about what can happen to people with Down syndrome and know about the wonderful things that can be done with early intervention, they also know there is no net out there to protect such children if and when the mother dies. The know what can happen to such children when there is no family and no money remains to guarantee the kind of support necessary. Not trusting this world, she terminates the pregnancy. It's not only about interpersonal communication. It's about economics and politics and structural issues. Great things can happen in the counseling, but it's not going to change the political economy.

Audience: Would there ever be a case where you would refuse to give genetic counseling? Take case number 4 for example, where if a fetus tested positive for the breast cancer mutation, the mother would abort. Would you refuse to do the testing?

Bluestone: I think that this issue also arises in the general medical context. A healthy person could come into your office convinced he has a tumor and say, "I want an MRI and a blood test and everything you have, just to make sure I don't have a tumor." Because none of those tests would guarantee that he doesn't have a tumor and if he's a normally healthy person with no risk factors, you wouldn't do the tests. If someone is very low risk but asks for every work up available, shouldn't we say no because it costs too much? All the testing available is not always going to provide all the answers you're looking for.

Audience: What if a physician has moral difficulty with providing services to a couple who are going to terminate because the fetus is carrying the gene for breast cancer and doesn't want to provide services to them.

Berkowitz: I can tell you, this already has happened relating to the issue of fetal gender. People have asked for an amniocentesis or CVS because they don't want a girl, or they don't want a boy. There are many places that do testing for gender selection. This kind of thing has been done, and is done.

Audience: We already have abortion on demand, so how can you refuse to do it based on gender selection?

Hirschhorn: You are quite right, we have abortion on demand, so how can we say that having the abortion for sex selection is the wrong thing? People say, "I want a girl" or "I want a boy," so half will abort instead of 100%. The cop out answer is, that these are expensive and valuable resources. They were set up to resolve more serious problems than whether the child was going to be a boy or a girl. We are very busy doing a lot of cases and using them for sex selection delays other cases that are high risk. That is unfair.

Berkowitz: There is a different answer that can be given based on the fact that physicians are not automatons. We are not obligated to do everything that a patient wants us to do. If a patient walks in and says, "I hate my left hand, take it off," I don't have an obligation to do it for him, and I wouldn't do it. I don't consider gender a disease, so I would not terminate a pregnancy for that as an indication. I realize we have abortion on demand and patients can walk into any place that provides that service, any day of the week and have an abortion for any reason. Still, I don't have to participate in it and I choose not to in this particular instance. I think physicians make these kinds of choices everyday. Although not all physicians would make the same choice, they do need to live with themselves and they do need to make choices like that.

Daniel Moros: I'm fascinated with the conflict between the health care community that visualizes its work on sick individuals and the much more radical critique with which Professors Katz Rothman and Mann are trying to challenge us. I wonder if this difficulty is related to the concept of the individual person. In her presentation Prof. Katz Rothman seemed terribly critical of the individual person who is not told properly, look there is a way of having such a child. You described a man left crying over the plight of his wife because he was not informed properly, and you seemed offended that, as an individual, he wasn't informed. Can you offer us a radical critique that asks, for example, in an overpopulated world, why worry about this?

Katz Rothman: In clinical medicine, seeing patients one at a time, I think we ought to be nondirective. I do think there is a difference between a counselor who says "I've had enough freaky kids throw up on me" and one who says, "There's a range of possibilities." I'm committed to the nondirectional kind of approach. I'm committed to the full disclosure of all the options and so on, that is absolutely necessary but absolutely not sufficient. The counselor would tell the person, "Yes, there was a great program in your neighborhood 5 years ago for kids with Down syndrome but they closed it down." She's not having the baby 5 years ago, she's having it now. Information is terrifically important and I'm not opposed to informed consent, I'm not opposed to as much autonomy as you can offer people. What I think we need to understand is that this all takes place within a larger context. If we keep pulling it down to the clinical individual level we end up with these incredible binds in which doctors think Down syndrome is tragic and a legitimate indication for terminating a pregnancy and, at the same time, think that a woman who might find herself out on the streets if she has a fourth daughter is making an unjustifiable choice. The consequences for that woman are the consequences for that particular woman. When you get down to the individual level you have to be nondirective, you have to have information about all the choices. You also have to step back and look at the political, social, and economic situations, the ideological context in which the choices are being made. They're not contradictions because they are different in scope. Within their scope, they're both true.

Mann: I think I would agree that the micro and the macro aren't so hard to bring together. I tried to bring this out in the terms that I was questioning how we define the social context. I think a cyborgian definition of the social context has a broader sense of what's there. In fact the traditional definition of a woman, the spouse wants to maintain his genetic line, the family, all this is the context. I don't think this is the only context, so I'm not certain about nondirectional counseling actually. I think the point is that in the real social context, we lack justification for providing these services in a purely instrumental vein commensurate with the view that women deserve to have children because they're women and families deserve to perpetuate themselves genetically because that's what families do. It doesn't make sense anymore and I don't think, on an individual level, it's a realistic social context either, at least ethically.

David Valone: I also want to respond to the notion that there is a miscommunication or that two different worlds are being presented here, a medical side and the radical critique, as Dr. Moros put it. I don't know if Professors Mann and Katz Rothman necessarily accept that label, but I certainly want to challenge it because, of course, these problems are not new. Mary Shelley's *Frankenstein* was basically about this issue, questioning what science controls and whether science changes our notion of what it is to be human. I don't think we need to be radicals to be nervous about living in a society in which we can't stand up and say that a selective abortion based on sex isn't a problem. It's then very easy to slide into accepting selective abortion based on the fact that the child is near-sighted. I think there's a lot of room for nervousness based on an appreciation of the humanistic perspective.

Moros: In the long run, Dr. Berkowitz is saying that he doesn't want to be involved with this application of the technology. This is not going to be an adequate solution for what needs to be a social decision. Are people going to be allowed to make this choice or not? I'm more on the side of Professors Katz Rothman and Mann. Sometimes we have to have an over-arching solution or decision about these things. In the long run it's just not adequate for society to leave it to the individual doctor to decide whether he or she wants to perform a procedure. I also think there is a sad result when selective abortion is performed in large numbers for sex selection.

Audience: But aren't you taking away our autonomy as physicians to practice in accordance with our own philosophies and beliefs, saying that society must decide these things? Perhaps that's adequate if the entire society is in agreement about some issue, but what if it's not? You're talking about global philosophy of the sort which lead humans from the Greeks to the Romans. But we're talking about day-to-day practice.

Mann: That's fine in the abstract but what if, for example, you want to rob a bank and your philosophy supports such an action?

Audience: I want to suggest that there is much more dialectic between the social context and what happens on a day-to-day basis in either the genetic counselor's office or the doctor's office. Dr. Hirschhorn mentioned that 3% of all births have abnormal outcomes and we've heard talk about lack of any kind of preparation on how to communicate this potential downside to prospective parents. We have a certain broad view of what's supposed to happen when a woman gives birth, which informs the way in which we train physicians. We don't train physicians to be prepared for the 3% of cases with bad outcomes. There is no woman who has a child with disabilities who hasn't had some physician say some very insensitive thing to her. I don't understand why in the training of physicians such issues aren't addressed given that 3% is a significant number. This is where the larger social interest should be informing the practice of medical training, especially of obstetricians and pediatricians. There's another issue that I'd like to give as an example. The question was raised about whether some people are going to have the support needed to be able to raise children with disabilities. Is the choice to not have such a child a selfish choice? How can one make the choice to have a child with Down syndrome or spina bifida knowing that the family will have to bear the entire burden of complications and the difficulties of raising such a child. Now when a genetic counselor counsels, it's perfectly possible to think those questions back into the discussion. It's not just a matter of whether or not she's going to have the choice but, as Professor Katz Rothman points out, it's important to consider the context in which she's going to have to make that choice and such considerations must be brought along with the technology. I think that's the question Dr. Rothman is trying to raise to the medical community. As we refine the technology and make it better, we must make that question of context bear on that technology and on the choices that will have to be made.

Katz Rothman: The first thing I'd like to point out is that doctors only took over births very recently. In the great historical scheme of things we're talking about the 1920s. When they took over their response to a child with a severe anomaly was to use general anesthesia to socially remove the mother so they didn't have to deal with her. The common practice was, if the baby was born with a cleft palate for example, the resident would knock the woman out, deliver the baby, go into the room later without the baby and have a little break-the-news session with the mother. Midwives had ways of dealing with it and still do, and we can learn from their experience. But we are now in this kind of interim world where the doctors can no longer say, "Oh dear lord, knock that woman out." The physicians have to deal with it. Having to revise the way these situations are dealt with has only occurred since the 1970s when it became unacceptable to use anesthesia for social control. They've only had to deal with this for the past 25 years and they haven't got it worked out yet. I think that's why there is no training. The other piece of it is that the policy level doesn't just concern disability. It's American social policy that every child is born into deep and grinding poverty unless it can find a particular adult with enough resources to pull it out. Some cultures have the notion that every child is born into its share of the communal pie and every child has equal access to the resources of the system. Most western European countries have had this view, and most tribal cultures have it. American society has it that every child is born into poverty unless it can hook onto somebody with the resources to pull it out for good. You can sue some guy for child support if he earns well and so take that child out of poverty. The more expensive the child is, the greater the resources the parents must have to pull the kid out. All of these decisions take place in this larger context of not taking communal responsibility for all of our children, including our healthy ones. Thus, amazingly small amounts of disability can toss the whole thing into the realm of the undoable.

Maria Bastillo: In my field we deal with this all the time. Part of the communication problem that I often see is that even if I try to make the broader context available to the patient, for example, explain third party reproduction, there is such a drive sometimes to reproduce their genetically related child, that what I say is not heard. I take great pride in the fact that I do try to put my explanation into the larger context of society. But it is very difficult when, for example, you are dealing with a couple who have experienced infertility for 10 years.

Hirschhorn: I can respond to the question which was asked earlier, why don't we teach our doctors to tell patients that there is 3% risk, or whatever. We do teach this. It's done in the genetics, obstetrics, and pediatrics courses. Then these physicians come out and face the situation of dealing with a women who is going to have a baby and they act as if the baby is normal until proven otherwise and do not want to rock the boat. There seems to be an unfortunate lack of response or understanding on the part of most of the medical staff and house officers. They either do not bear such risks in mind or do not feel comfortable discussing the issue of risk with a couple when pregnancy has already occurred.

Audience: I didn't mean that those statistics are not taught. But I'm asking why medical institutions don't teach people how to deal appropriately with the parents of a child that is born with some sort of abnormality.

Bluestone: I was taught all those things but it doesn't change those first few moments in a delivery room, the first encounter with that situation. When you first see such a child, it's still a shock and it's still tough. I don't say anything like, "Boy, your baby's ugly!" I try to be as sensitive and caring as I can be. Nonetheless, the situation is still traumatic no matter how much training you've had. Of course it's worse for the mother, but you try your best and that's the best you can do.

Stefan Baumrin: I don't see, Professor Rothman, why it's the physician's responsibility to remedy society's ills. And if it isn't then I don't understand the challenge to them. Why should they be put on the spot because society doesn't provide for a different view of parenting or a different view of disability? Also, it's not just that babies come into the world poor. People decide to have babies. Some are rich parents and some are poor parents. It's not the society that defines the child's wealth status.

Katz Rothman: That's not quite true. A child born in Sweden is guaranteed a measure of support that a child born into the South Bronx is not. That's not a matter of the parents' choice. That's a societal choice about the support of children. It's not that I'm holding genetic counselors responsible but if we're going to have a discussion, lets go back to Sophie's choice. If you were going to have a meeting on issues in Nazi Germany and you wanted to discuss ethical issues, counseling for Sophie would not be the prime issue. I've been to many conferences on the ethical concerns of genetics and the ethical concerns of informed consent, confidentiality, autonomy in decision-making, and rarely do we move beyond that to the ethical concerns about societal structure. I don't think ethics is something that exists just in the doctor-patient relationship. I think there are ethical and unethical relationships between societies and the people who live in them. If we are not going to discuss the larger ethical context of genetic testing and reproductive technology at a conference on the ethics of genetics and reproductive technology, then there really isn't much of a venue for it. I think it is necessary to do the stuff we've talked about, at the individual level; it's very important, but we must also devote a certain amount of our time to an understanding of the larger context.

Berkowitz: You mention Sophie's choice, but Sophie didn't have a choice about having a choice. You started off by saying that choice isn't necessarily terrific. That's right. It isn't always terrific, it isn't always liberating, it isn't always wonderful. But there are things that happen, and we have to make choices. One arena in which we have to make choices is whether or not we want to know if there is an abnormality in a fetus. Now if you choose not to know, I think that's fine. No one is forced to have a screening test, no one is forced to have an ultrasound examination, no one is forced to have an amniocentesis. People choose to be tested because they want to know whether their fetus is normal or abnormal. Our technology is far from perfect. It doesn't answer every question and our technology makes mistakes. But the issue for an individual is whether or not they would like to know. Many people in our society want to know. What do we do with those people? That's why I don't quite understand your position. You said you are not for screening and you are not against screening. So what are you for?

Katz Rothman: You are completely right at the level of the individual. Nobody is forced to make the choice to get or not get the information. However, often the women who go through this use language that doesn't sound a lot like choice. An expression you frequently hear from women is “My only choice. . .” Saying this means the situation they experience is one in which they make a choice but in fact don't really have a choice. There's really only one thing they can choose. For example, perhaps they don't have a lot of brothers or sisters or a large family, no other kids, and so will be the sole support for this child until death and after that the kid is just up a creek. In such a case the only choice is to learn whether the fetus is going to be abnormal. So the woman chooses to have the testing and then, if necessary, have the abortion now so the child won't suffer when it's thirty. A choice is being made at the level of the individual and those counseling such women should absolutely give them all the information, expand choices, but you have to understand that the choice is often made in a context an individual didn't choose.

Mann: Let me add to that. This question concerning what are we asking of the medical community is important. The position of power that medical professionals have in providing service and also in providing information about such services means the idea that one neutrally can provide information or be completely nondirectional is flawed. Physicians are in a position of offering something that has great value—physicians value it, the culture values it, and people come to you for it. The implication of what we're saying from different perspectives is that perhaps part of your responsibility is to offer more information, offer different information, become more conscious of what sort of directional force you have, and perhaps to have a different orientation with respect to arguing against the need to perpetuate a family genetically. Maybe, in fact, the ethical position that you, as those offering the service should take, is telling people that it's inappropriate in many cases but this hasn't been worked out. Given that the culture is pushing a certain way and the value of technology is pushing a certain way, it might be that what's appropriate in response to these amazing technologies is to advise people not to use them in many cases and, if they decide to use them, to offer advice.

Berkowitz: Let me frame my concern in a different context. Within the uterus of a woman who is pregnant there is a fetus. That fetus does or does not have a problem, say Tay-Sachs disease. It either has it or it doesn't. Now the question concerning the mother is, does she want to know or does she just want to let nature take its course. From the standpoint of the mother, father, and the physician, that's the question. The technology has nothing to do with whether that disease is present or not. The technology can be in error, the counseling can be lousy, there are a million things that can go wrong. But the essence of this issue is either that fetus does or does not have a particular abnormality. The question that we seem to be talking all around is, does the mother want to know? If she does, we're here to try to help her find out. We're not always right, but that's what the dialogue between the patient and the doctor is all about. Society may have millions of things wrong with it, but in an individual pregnancy, that's the question.

Katz Rothman: I'm really not arguing against that. I think that good genetic counseling, which is probably 99% of all genetic counseling, is a fine thing and provides a service. I'm not objecting to it, I'm only concerned that by keeping our eyes so close to the field or focused only on the clinical scene, we lose the larger context. We have data for instance that make it clear that people have a lot of repugnance to a full range of disabilities which may or may not necessarily match with their doctor's repugnance. People are horrified by fat, there is a lot of homophobia, so a woman might desperately want to know if her child is going to be a 300-pound lesbian or not because that would be a totally intolerable condition for her. I suppose I'm on the side that says she has a right to know and to provide the counseling and information, etc. At the same time I ask what kind of world is it in which a 300-pound lesbian is a tragedy? Go ahead with counseling. I'm on the side of more information and more choice. However, I have real trouble with saying I won't terminate on the basis of sex although I have real trouble with the idea of aborting on the basis of sex, but don't just stop there. The discussion isn't a clinical problem, it goes beyond what is happening clinically.

Audience: I'm a little surprised that those on the medical side of the panel are having trouble seeing the position in which women are put. It's not a matter of getting rid of the counseling which does a lot of good but rather also taking some responsibility for the kinds of positions in which women are put. What about a poor woman who can't afford the counseling and testing and so it's a choice between aborting, which might horrify her, or putting her child in this terrible position of not being well looked after, which might horrify her even more. More reflection on the whole practice is a good thing.

Audience: Let me switch gears and ask Dr. Berkowitz a question. What should be done about this epidemic of multiple births? How should technology change?

Berkowitz: That's an excellent question. In addition to not living in Sweden with their enlightened social policy, we also do not live in a society which regulates its physicians. In countries that have national health service there is control over how physicians practice, how reimbursement flows. There have been policy decisions which have been made centrally which have been made about how health care is practiced. We don't have that and I think that there are true abuses of the technology that we have access to in this regard. I think many responsible people in the reproductive and endocrine community agree with that. We will probably never be able to eliminate the increase in triplet pregnancies that now exists because even with the very best monitoring techniques available, we simply won't be able to prevent them. Still, there is no good reason for us to be seeing so many quads and higher numbers. To give an example, I just saw a patient who came in with six fetuses, after having undergone IVF in a private practice setting. I asked how many embryos were transferred and the answer was 12. Now that, as far as I'm concerned, is unacceptable and wrong by any definition you could use. It's just unacceptable. But at this time we have no central regulating agency. There is no question that some abuses are being perpetrated.

Bastillo: A major impetus behind implanting a lot of embryos is to increase the pregnancy rate. So this is probably the biggest argument in favor of two things that I'm very much for. One is coverage for this procedure so it's not everyone grabbing for a set pool of patients and not only a technique for the elite but for whoever needs it. Second, it is a very good argument in favor of human embryo research. If we were able to know ahead of time that one embryo had a greater chance of implanting than another, there would be a scientific means to limit or somewhat control the number of multiple fetuses.

Rhodes: Dr. Bastillo and Dr. Berkowitz have been talking about medicine regulating medicine. Dr. Hirschhorn was talking about an ethics committees regulating medicine. Who should be regulating medicine or these reproductive technologies?

Hirschhorn: We're part of society so it's a dialogue really among the political elements of society, the medical elements, and the regulatory governmental parts of society that have to sit down together and work out what's right. This isn't happening and isn't likely to happen very soon. In fact, if anything it's getting worse and people are talking past each other rather than to each other. Nothing very good is coming out of this. Even the good governmental regulation aspects are being politicized.

Willner: Obviously, it's not a question I've thought of except in terms of what's happening currently. Currently the insurance companies are regulating us. I don't think the insurance companies should be regulating physicians. The future of health care in this country is insurance companies taking control away from physicians and, to some degree, from government regulations. I think we're now seeing some response to this from society, which is encouraging.

Elmer's Glue, Elsie and You:

Clinical Applications of Adhesion Molecules

Lynn M. Schnapp, M.D.

Abstract

An effective response to injury or inflammation requires leukocyte migration from the endovascular compartment into areas of inflammation. This process requires the appropriate expression of adhesion molecules, which mediate cell-cell and cell-extracellular matrix interactions. A better understanding of the roles of adhesion molecules in normal and pathological conditions might lead to the development of more effective therapeutic interventions. Features of several classes of adhesion molecules, including immunoglobulin superfamily, selectins, and integrins, are reviewed along with how they participate in leukocyte-endothelial interactions. The role of cell adhesion molecules in a variety of pathological conditions, including graft rejection, reperfusion injury, and acute lung injury are discussed. In addition, some recent studies that explore therapeutic uses of adhesion molecules are summarized. **Key Words:** Integrins, adhesion molecules, leukocytes, clinical applications, review.

Many processes that we generally encounter in clinical medicine, such as infections, graft rejection, and ischemia, are characterized by accumulation of leukocytes in involved areas. Although this is often an appropriate response, leukocyte influx may result in more damage than the initial insult. How do leukocytes travel from the endovascular compartment into the interstitial spaces? What are the molecules involved? Understanding the steps involved in these processes will allow us to develop therapeutic modalities that may ameliorate some of the damage that occurs. This review will address the roles of leukocyte adhesion molecules in normal and pathological conditions and review some therapeutic modalities based on adhesion molecules.

Cell Adhesion Families

To understand leukocyte interactions with endothelial cells, we need to familiarize ourselves with different classes of cell adhesion molecules.

Immunoglobulin Superfamily

The first class consists of members of the immunoglobulin (Ig) superfamily. This is a large family of related molecules which contain one or more Ig-like domains and functions in cell-cell adhesion (Fig. 1). Members of the immunoglobulin superfamily include the intercellular adhesion molecule-1 (ICAM-1), the vascular cell adhesion molecule-1 (VCAM-1), and the mucosal addressin cell adhesion molecule (MadCAM) (Table 1). These molecules play a role in both adhesion and migration of leukocytes. Several members of this family are ligands for integrins. ICAM-1 is found on a variety of cells, including endothelial cells, T cells, and B cells, and is a ligand for β2 integrins (1). ICAM-1 expression on endothelial cells is induced by a variety of cytokines such as tumor necrosis factor (TNF), interleukin-1 (IL-1), and endotoxin (5). A related molecule, ICAM-2, is also a

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Address correspondence to Lynn M. Schnapp, M.D., Assistant Professor, Division of Pulmonary and Critical Care Medicine, Department of Medicine, Box 1232, Mount Sinai School of Medicine, One East 100th Street, New York, NY 10029.

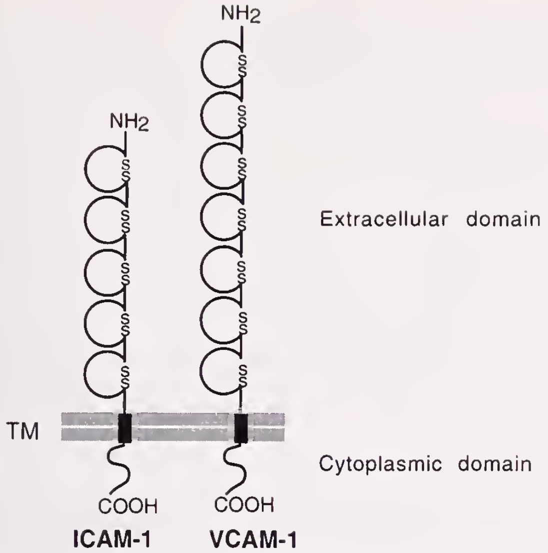

Fig. 1. Schematic drawings of the intercellular adhesion molecule-1 (ICAM-1) and the vascular cell adhesion molecule-1 (VCAM-1). The extracellular domain of each molecule consists of several immunoglobulin-like repeats folded into a loop by disulfide bonds, followed by a transmembrane domain (TM) and a short cytoplasmic tail.

ligand for β2 integrins (6). In contrast to ICAM-1, ICAM-2 appears to be expressed constitutively on endothelial cells. VCAM-1 interacts with α4-containing integrins and plays a role in the adhesion and migration of lymphocytes, as well as eosinophils and basophils. MadCAM plays a role in the homing of lymphocytes to Peyer's patch high endothelial venules in the gut (7).

Selectins

Selectins are a group of cell surface molecules which are important in the regulation of leukocyte trafficking (Table 2). They are characterized by a lectin-binding domain on the amino-terminus of the molecule, an epithelial growth factor (EGF)-like domain, and complement regulatory-like repeats (8, 9) (Fig. 2). The highly conserved lectin domain binds to specific oligosaccharides, such as the sialyl Lewis X antigen (Sle^x), on the surface of another cell (10–12). L-Selectin (LAM-1, MEL-14) plays a key role in lymphocyte homing to high endothelial venules. L-Selectin is expressed on leukocytes, including neutrophils, where it is shed from the surface after activation and before migration. E-Selectin (ELAM-1) is expressed on endothelial cells only after activation and plays a role in leukocyte adhesion. P-Selectin (GMP 140, PADGEM) is found in platelets and endothelial cells. P-Selectin is stored in the membranes of Weibel-Palade bodies in endothelial cells and α-granules in platelets. After cell activation, P-selectin can join the plasma membrane and can mediate platelet-leukocyte and leukocyte-endothelial cell interactions.

Integrins

The term integrins was first coined in 1987 by Richard Hynes to designate a family of receptors that seems to "integrate" the cytoskeleton of one cell with that of another cell or extracellular matrix protein (13). An integrin is a heterodimeric glycoprotein that is composed of two noncovalently associated subunits, α and β (Fig. 3) (for review, see Ref. 14). Each α and β subunit contains a large extracellular domain, a transmembrane domain, and a cytoplasmic domain; the latter presumably interacts with the cytoskeleton and/or cytoplasmic signaling molecules. These extracellular domains of both subunits form the ligand binding site for different substrates such as fibronectin, collagen, and laminin. Many integrins recognize the tripeptide sequence Arg-Gly-Asp (RGD) found in matrix proteins including fibronectin, vitronectin, and fibrinogen (15). Integrins may exist in different conformations which can be regulated by cytokines, agonists, or cations.

Integrins can be divided roughly into subfamilies according to their shared β subunit (Fig. 4). More recently, it has been recognized that α subunits sometimes can bind to more than one β subunit, thus blurring the original subfamilies. The β1 subfamily of integrins was originally termed VLA (very late activation) antigens because they originally were detected on T cells "very late after" mitogen stimulation (16). The β2 integrins are found exclusively on leukocytes (17, 18) (Table 3). The β3 subfamily includes the platelet integrin αIIbβ3 (GPIIb/IIIa) and the vitronectin receptor, αvβ3.

Leukocyte-Endothelial Interactions

Neutrophil interaction with endothelium can be divided into several steps (Fig. 5) (for review, see Ref. 19):

1. Rolling: Neutrophils roll along the endothelium through selectins (E-selectin on leukocytes with its carbohydrate ligand on endothelium, and P- and E-selectins on activated endothelium with their ligands on leukocytes). If no activation occurs, the leukocytes detach and continue rolling along the endothelium.
2. Activation: If chemoattractants are present (i.e., interleukin-8, chemokines), then the β2

TABLE 1
Immunoglobulin Superfamily

Receptor	Distribution	Ligand	Ref.
ICAM-1	Endothelial cells, hematopoietic cells, others, induced by inflammation	αLβ2, αMβ2	1
ICAM-2	As above, except constitutive expression	αLβ2	
VCAM-1	Activated endothelial cells, smooth muscle cells	α4β1, α4β7	2,3
MadCAM	High endothelial venules	α4β7	4

TABLE 2
Selectin Family

Receptor	Distribution	Ligand
L-Selectin	Leukocytes	Endothelial cell carbohydrate
E-Selectin	Activated endothelial cells	Leukocyte carbohydrate
P-Selectin	Platelets, endothelial cells	Leukocyte carbohydrate

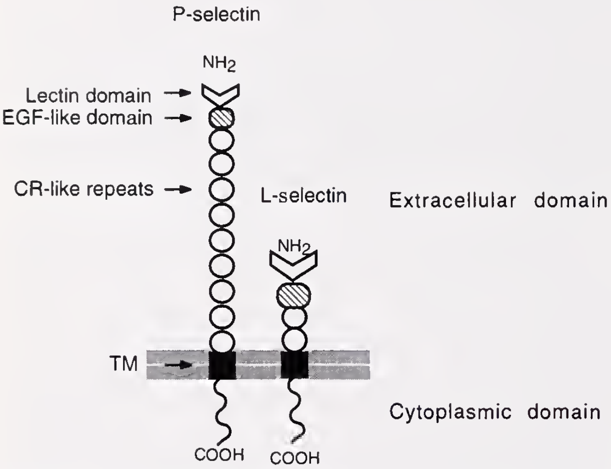
P-selectin
NH2
Lectin domain
EGF-like domain
CR-like repeats
L-selectin
NH2
Extracellular domain
TM
Cytoplasmic domain
COOH
COOH

Fig. 2. Schematic drawing of P-selectin and L-selectin. EGF, epithelial growth factor; CR, complement regulatory; TM, transmembrane domain.

integrins on the leukocytes become activated, which allows arrest.

3. Arrest: Firm adhesion of the leukocytes to the endothelium via β2 integrin-ICAM-1 interactions is a necessary prerequisite for the transmigration of the neutrophil into the interstitium.

Our knowledge of leukocyte-endothelial interactions has been advanced largely by the use of intravital microscopy. Once an animal is anesthetized, the mesentery (with readily visible venules) is laid across the stage of a specially prepared microscope and maintained under physiologic conditions for the duration of the experiment. This allows direct visualization of leukocyte movement under various conditions. Regulated expression and function of endothelial-leukocyte adhesion molecules are necessary for the proper development of an inflammatory response. This makes them attractive candidates for the development of therapeutic interventions.

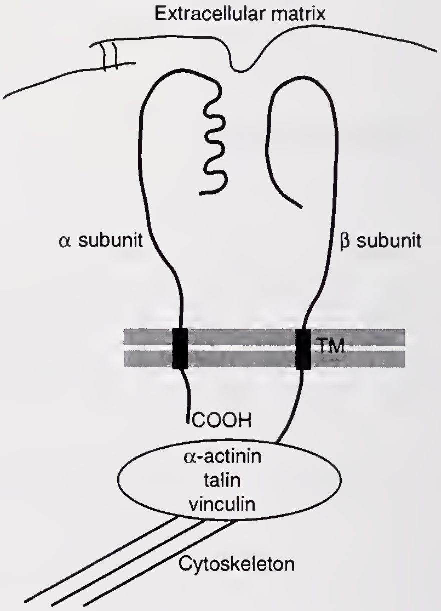
Extracellular matrix
α subunit
β subunit
TM
COOH
α-actinin
talin
vinculin
Cytoskeleton

Fig. 3. Schematic drawing of an integrin. The integrin is composed of two subunits, α and β. The extracellular domain binds to matrix proteins. The short cytoplasmic domains interact with cytoskeletal molecules which link the integrin to actin cytoskeleton. TM, transmembrane domain.

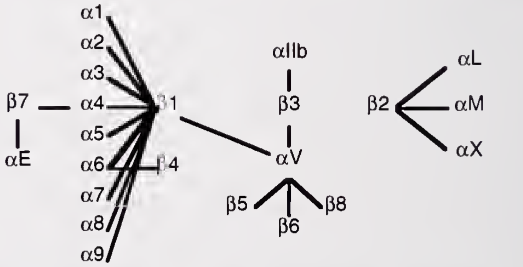
α1
α2
α3
α4
α5
α6
α7
α8
α9
β1
β4
β7
αE
αIIb
β3
αV
β5
β6
β8
β2
αL
αM
αX

Fig. 4. Integrin subfamilies, illustrating the different subunit associations.

Leukocyte Adhesion Deficiency Syndrome

The importance of adhesion molecules in normal homeostasis is illustrated by several disorders involving defects in expression of cell adhesion molecules. Leukocyte adhesion deficiency

TABLE 3

β2 Subfamily, Leukocyte Integrins: Nomenclature and Ligands

Leukocyte Integrin	Names	Ligands[a]
αLβ2	LFA-1, CD11a/CD18	ICAM-1, ICAM-2, ICAM-3
αMβ2	Mac-1, CD11b/CD18, CR3	ICAM-1, iC3b, Factor X, LPS, Fibrinogen
αXβ2	p150,95, CD11c/CD18	iC3b, Fibrinogen

[a] iC3b, inactivated complement 3b fragment; LPS, lipopolysaccharide.

(LAD-1) is a rare, autosomal recessive disorder in which patients suffer from recurrent, severe bacterial infections (20). Leukocytes (especially neutrophils) are unable to migrate to tissue sites of inflammation and infection despite normal to high numbers of circulating leukocytes. The inability of the leukocytes to migrate is caused by a quantitative or qualitative defect of β2 integrins on leukocytes. This disorder illustrates the importance of integrins in the normal host defense of infections.

In 1983, reports appeared of Holstein cows experiencing recurrent infections, periodontal gingivitis, recurrent pneumonia, and neutrophilia. These cows eventually were diagnosed with a deficiency in the β2 integrin subunit expression, the bovine equivalent of leukocyte adhesion deficiency (BLAD) (21). The incidence of BLAD in Holstein cows was 0.2% (similar to the incidence of cystic fibrosis in humans), making BLAD the most prevalent genetic disease in cows (22). The economic impact of this disease is impressive. In the United States, 80% of dairy cows are Holsteins. Therefore, approximately 16,000 calves are born with BLAD each year, at a loss of approximately $300/calf, costing the dairy industry approximately $5 million/year, making BLAD one of the most costly genetic diseases in animal agriculture (22).

In contrast to the human disease, in which multiple mutations in the β2 subunit have been described, all affected cows carried the same point mutation. Through analysis of breeding records, it was determined that all affected cows were related and could be traced to a single bull, Osborndale Ivanhoe (b. 1952). Because of his high genetic merit for milk production, Osborndale Ivanhoe sired thousands of offspring around the world. Known as the "Father of the Holstein Breed," he is the animal with the greatest genetic relationship to the Holstein breed in the United States today. Testing of his frozen semen determined that he was indeed a carrier of the point mutation for BLAD (22). Schuster et al. (22) devised a screening technique for the trait based on restriction fragment length polymorphism (RFLP). Now, all bulls are screened for this trait and the incidence of BLAD should decline dramatically. This was the first worldwide attempt to select animals for a specific DNA sequence.

More Than Just Glue

Adhesion Molecules and Graft Rejection

αLβ2 (LFA-1)/ICAM-1 interaction is important for many T-cell functions. These include the response of T cells to antigen-presenting cells (APC), enhancement of lysis of target cells by both NK cells and cytotoxic cells, and immunoglobulin production through interactions of the T cells with B cells (23, 24). Several lines of indirect evidence suggested that αLβ2/ICAM-1 interactions play a role in graft rejection. For example, during acute cardiac rejection, ICAM-1 expression increases on vascular endothelium and is induced on myocytes and endocardium. The increased expression of ICAM-1 correlates with increased leukocyte infiltration (25, 26).

How might αLβ2/ICAM-1 interactions contribute to rejection? The interactions may facilitate migration of leukocytes into cardiac tissue. αLβ2/ICAM-1 interactions may increase the strength of adhesion between a T cell and APC or target cell, and allow time for additional functional interactions. Another possibility is that αLβ2/ICAM-1 interaction might provide a co-stimulatory signal required for T-cell activation and clonal expansion. Thus, continuous stimulation of alloantigens in the absence of αLβ2/ICAM-1 signals might lead to the induction of specific tolerance. Experiments have been carried out to assess more directly the role of αLβ2/ICAM-1 interactions in graft rejection.

Isobe et al. (27) examined survival of allogenic heart transplants in mice. Animals treated with a daily dose of either anti-αL or anti-ICAM-1 antibody for 6 days after transplant showed prolonged graft survival (50 days versus 10 days). Animals treated with both anti-αL and anti-ICAM-1 antibodies showed indefinite graft survival, confirmed by the absence of active graft rejection histologically. Strain-specific tolerance was also induced in these mice as shown by the acceptance of donor syngeneic grafts and rejection of third party grafts. A similar study showed that

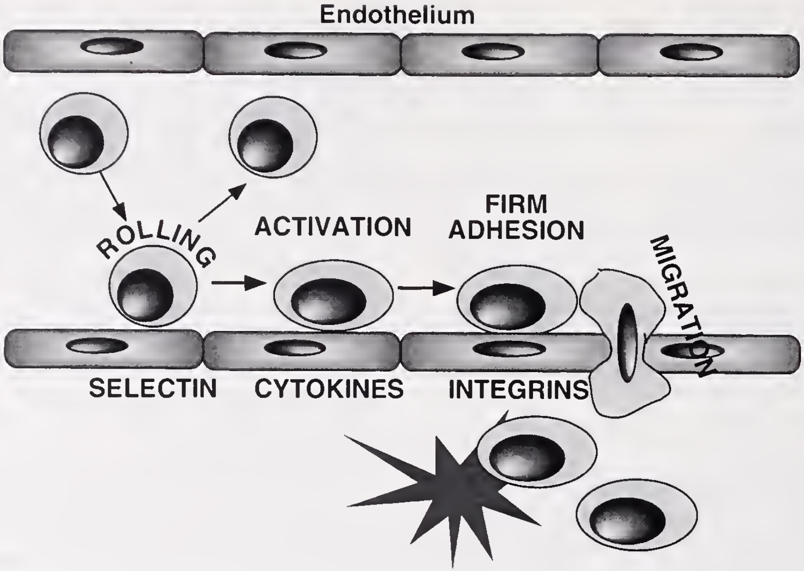

Fig. 5. Multistep model of leukocyte-endothelial interactions. The initial rolling of leukocytes along the endothelium is mediated by selectins. After activation by cytokines, integrins mediate firm adhesion followed by transmigration of leukocytes to areas of inflammation.

IV infusion of both anti-sense ICAM-1 and anti-αL antibody resulted in indefinite survival of an allogenic transplant in mice. Donor specific tolerance was also confirmed (28).

Clinical trials in high-risk kidney transplant patients showed that patients treated with anti-ICAM-1 antibody (given in concert with cyclosporine A, azathioprine, and steroids) resulted in improved 1-year allograft survival (56–78%). However, 16 of 18 patients developed anti-mouse antibodies, thereby limiting the repeated administration of the antibody (29). These studies and others suggest that the αLβ2/ICAM-1 interaction plays a role in graft survival.

The availability of transgenic animals has led to additional studies examining the role of molecules in graft rejection. Genetically engineered mice that lack ICAM-1 expression were used as either donors or recipients of incompatible hearts (30). In contrast to the antibody studies, no difference in allograft survival was noted.

Cell-mediated immunity in animals with BLAD was studied by skin transplantation experiments (31). Skin allograft survival in animals with BLAD was compared with the behavior of simultaneously transplanted allografts in healthy controls. Allograft survival time was prolonged in three BLAD cattle (28, 30, and 72 days) compared with six healthy controls (12–14 days). When transplantations were repeated on one animal with BLAD using skin grafts from the same donor, accelerated rejection was observed (allograft survival time decreased from 72 days at primary to 35 days at secondary and to 21 days at tertiary transplantation), suggesting the development of immunological memory. Graft-infiltrating lymphocytes that were obtained from allograft biopsies during the period of rejection were shown to be of recipient origin (β2 integrin negative). Thus, although prolonged allograft survival is observed in cattle with BLAD, skin allografts are ultimately rejected.

There are several possible explanations for the differing results in antibody-induced blockade of molecules and genetically engineered loss of molecules. It is possible that antibodies are inducing additional changes in endothelial cells (through interactions with the targeted molecule). Differences also may be caused by the time course. Genetically engineered mice may also allow expression of alternative pathways. For example, other related members of the immunoglobulin superfamily (i.e., ICAM-2, ICAM-3) may compensate for the loss of function of ICAM-1.

Ischemia/Reperfusion Injury

Early re-establishment of coronary circulation after ischemia is essential for tissue preservation. However, reperfusion itself may cause additional injury. Lucchesi (32) reviewed previous studies which showed that neutrophils play an essential role in postischemic/reperfusion injury. Proposed mechanisms of neutrophil-induced injury include generation of oxygen radicals by neutrophils [binding of αMβ2 on neutrophils to

ICAM-1 on myocytes initiates free radical formation (33)], coronary microvascular capillary plugging, and release of proteolytic enzymes. Adhesion to endothelium is a critical step in the extravasation of neutrophils and their subsequent binding to, and injuring of, cardiac myocytes. Youker et al. (34) have documented interventions of β2 integrins on neutrophils with ICAM-1 on endothelium and cardiac myocytes and showed that ICAM-1 expression on cardiac myocytes is induced during reperfusion.

Multiple studies have examined the role of integrin-ligand interactions in ischemia/reperfusion. Hartman et al. (35) examined the effect of anti-ICAM-1 antibodies in a canine model of reperfusion injury. They found that administration of ICAM-1 antibodies decreased both infarct size and myeloperoxidase activity. In a study examining reperfusion injury in isolated ischemic hearts, antibodies to αL or ICAM-1 administered just before reperfusion resulted in improved indicators of left ventricular function and less edema when compared with controls (36). Another study treated the perfusate with anti-β2 antibody before reperfusion. The β2 antibody-treated group had improved cardiac pump function despite similar coronary flow rates and leukocyte retention rates when compared with the control group. This suggests that interference with the inflammatory process, rather than differences in leukocyte migration or capillary plugging was important in injury (37). Thus, it appears that disruption of the β2 integrin-ICAM-1 interaction may ameliorate injury associated with reperfusion.

Acute Lung Injury

Leukocyte migration in the pulmonary circulation differs from leukocyte migration in the systemic circulation in several ways. In the lungs, the primary site of leukocyte migration is the pulmonary capillary, whereas in the systemic circulation, the site of migration is the postcapillary venule. The diameter of the pulmonary capillaries doesn't allow rolling of leukocytes. Instead, it has been proposed that the architecture of the pulmonary microvasculature allows for mechanically induced contact of neutrophils and endothelial cells, which can substitute for the "traditional" rolling of neutrophils in systemic circulation (38). In the lungs, both β2 integrin-dependent and β2 integrin-independent pathways exist, whereas in the systemic circulation, all neutrophil migration is β2 integrin-dependent (Table 4). In addition, both selectin-dependent and -independent pathways exist in the lung which allow neutrophil migration from the pulmonary circulation (44). The nature of the initial insult also influences the molecules involved in neutrophil migration (Table 4). Another consideration in the lung is the compartmentalization of adhesion molecules. For example, in a model of immune complex-induced lung injury, intravenous, but not intratracheal, administration of anti-αL antibodies was effective in decreasing lung injury (45). These authors also reported that intratracheal but not intravenous administration of anti-αM antibodies was effective in decreasing lung injury (45). Freeman et al. (39) showed that a monoclonal antibody directed against the β2 subunit was effective in reducing *Escherichia coli*-induced lung injury in rats. However, the use of monoclonal antibody therapy also correlated with increased mortality. Therefore, although lung damage was ameliorated, impaired host defense presumably caused by antibody therapy led to decreased survival.

TABLE 4

Integrin Requirements for Development of Acute Lung Injury

Model	β2 Integrin-dependent?	Comments	Ref.
E. coli	Yes		39
Pseudomonas aeroginosa	Yes	Acute	40
Streptococcus pneumoniae	No	Prevented peritonitis	41
IgG	Yes	αL > αM	42
IgA	Yes	αM > αL	42
Cobra venom factor	Yes		42
Hydrochloric acid	No	Remote injury is β2 integrin-dependent	43

Summary

Cell adhesion molecules clearly play an important role in normal homeostasis and in diverse disease processes. There is a great deal of interest in developing therapies designed to interfere with endothelial-leukocyte adhesion. Additional studies using monoclonal antibodies, antisense constructs, and peptide analogues currently are being conducted. Adhesion molecules not only serve as "detectors" of the extracellular environment, they also transmit signals that affect cell behavior. As our understanding of cell adhesion molecules improves, we may be able to design more effective therapeutic modalities.

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A Personal Reflection on a Time Past

Mark M. Imberman, M.D.

It was 1935. I was in training in a prestigious hospital in Vienna, Austria, and on duty for admission. A woman was brought in by ambulance by her husband. He gave the following story. His wife had been ill for a few days with shaking chills and fever, her face had become swollen and reddish, her eyes were closed, she did not speak. After examining her, I concluded that she had erysipelas, a streptococcal infection of the skin with lymphatic spread. One must realize that this was a time before antibiotics.

While wondering what to do for her, I remembered that earlier the same day a detail man of I. G. Farben (Bayer) had spoken to me about a new therapeutic agent, Prontosil. It was the first of a group of azo-compounds, a forerunner of the sulfa drugs. It was a reddish liquid in a 20-mL ampule. He had told me that it had to be administered carefully intravenously because extravasation caused tissue necrosis.

He left a few ampules for me and told me that Dr. Domagk, a German pathologist and bacteriologist who did research at I. G. Farben, had found that the material exhibited therapeutic properties against streptococcic and staphylococcic infections. The compound contained an azo dye called sulfonamide and was said to have a low toxicity. The detail man also told me that Dr. Domagk had administered the drug to treat his own daughter when she was seriously ill with a staphylococcal infection.

I remembered having read about the new drug in a medical journal, but there was no extensive experience. I was not sure what to do with respect to treating my patient with this new drug, so I called the head of the department for guidance. He was of no help. He left it entirely up to me to do what I thought best. Because I could offer no known effective treatment for this patient's condition, I decided to proceed and inject one ampule of Prontosil intravenously. I was very nervous and said a prayer for my patient and me.

The next day, when I went to see the patient, her temperature was normal. She opened her eyes and wondered where she was and what had happened. Everybody in the hospital came to see her. I received many calls about her and the drug. A few days later, when her husband was taking her home, he thanked me for all that I had done, and asked when I was planning to go into private practice.

When I opened my private practice soon thereafter, the husband and wife became my patients. The husband asked me whether I would be interested in becoming a house physician in one of the theaters in Vienna, the Theater An der Wien. He was a theatrical agent and knew that such a position was vacant. The Theater An der Wien was the second oldest theater in Vienna. Beethoven's Fidelo was first performed there in 1805 and Beethoven had lived in a small apartment in the rear of the building. Many plays and operettas were performed there. There was a city law in Vienna that every theater had to have a house physician who had to be assigned an aisle seat in the orchestra. He was required to make rounds backstage half an hour before curtain time. He also had to be available during the performance in case of an emergency in the audience. I learned a great deal about stage fright and met many actors and singers. I was able to build a nice practice.

In March 1938, Hitler annexed Austria to Germany. Even though many of my patients were highly protective of me, I was told to leave Vienna quickly because something dreadful was going to happen. I left Vienna with my wife on November 9, 1938, the Kristallnacht (crystal night), when

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Address correspondence to Mark M. Imberman, M.D., 1080 Fifth Avenue, New York, NY 10128.

the synagogues were burned. Many Jews were rounded up and sent to concentration camps. We were on the last train to go through Austria and Germany into Holland before the border was closed. The next day we learned what had happened. We arrived in New York, November 23, 1938, the day before Thanksgiving, a day which has had a special meaning for me ever since. I took my boards in English and Medicine in 1939. Dr. Gerhard Domagk received the Nobel prize in 1939 for physiology and medicine for his discovery of the antibacterial effect of Prontosil. I joined the medical staff of The Mount Sinai Hospital in 1940. I have been a member of the faculty and Director of Student Health until my recent retirement.

Announcement of 1997 Awards

The Mount Sinai Journal of Medicine offers several prizes for manuscripts published in *The Journal* during the previous year and judged by an independent Awards Committee to be outstanding contributions to the medical literature. The committee's task certainly was formidable, but as expected, I believe that it has chosen wisely and well. The efforts of the members are much appreciated.

The 1997 Ralph Colp Award honors the memory of a distinguished Chief of Surgery at The Mount Sinai Hospital. It is for the best paper on a surgical topic. The chairperson of the Awards Committee informed me, after a prolonged discussion and many secret ballots by the members of the committee, that they were unable to select a single paper from the many which had been nominated. Therefore, the committee recommended that two papers be designated as recipients of the 1997 Colp Award. I am presenting them in chronologic order of their publication. The first award goes to Yun Peng Huang, M.D.; Toshio Okudera, M.D.; Akio Fukusumi, M.D.; Fumiaki Maehara, M.D.; Aryeh L. Stollman, M.D.; Roger Mosesson, M.D.; Mike Lidov; and Harold A. Mitty, M.D., for their paper entitled "Venous Architecture of Cerebral Hemispheric White Matter and Comments on Pathogenesis of Medullary Venous and Other Cerebral Vascular Malformations," Volume 64, No. 3, pp. 197–206. The second award goes to Salvatore Silvestri, M.D., and Susan Aronson, M.D., for their paper entitled "Severe Head Injury: Prehospital and Emergency Department Management," Volume 64, Nos. 4 & 5, pp. 329–338.

The 1997 Globus Award, established in honor of the first and long-time editor of *The Journal,* is for the best overall paper on a clinical topic. The award goes to Harry Spiera, M.D., and Robert F. Spiera, M.D., for their paper entitled "Silicone Breast Implants and Connective Tissue Disease: An Overview," Volume 64, No. 6, pp. 363–371.

On behalf of the Editorial Staff, I am delighted to recognize the outstanding scholarship and contributions of our 1997 awardees to medicine in general, and to *The Mount Sinai Journal of Medicine* in particular.

Sherman Kupfer, M.D.
Professor of Medicine
Physiology and Biophysics
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CONTENTS

GRAND ROUNDS

Money, Medicine and Methuselah *Christine K. Cassel* 237
New Concepts in the Pathogenesis and Treatment of Allergic Asthma *Mitchell H. Grayson, and Bruce S. Bochner* 246

GENERAL ARTICLES

Current Events and Bioethical Concerns in Physician-Assisted Death *Jeffrey T. Berger, Fred Rosner, and Allen J. Bennett* 257
Anesthesia for Nonobstetric Surgery During Pregnancy *Yaakov Beilin* 265
Concepts of Preemptive Analgesia for Postoperative Pain *Medhat R. Wassef* 271
Multiple Cerebral Infarction in Japanese Breath-Hold Divers: Two Case Reports *Kiyotaka Kohshi, Yoshimasa Kinoshita, Haruhiko Abe, and Toshio Okudera* 280
Severe Hyponatremia, Neuroleptic Malignant Syndrome, Rhabdomyolysis and Acute Renal Failure: A Case Report *Cristina Elizalde-Sciavolino, Alex Racco, Tamie Proscia-Lieto, and Morton Kleiner* 284
Complications of Subcutaneous Injection Ports *Francis A. Dysarz, III, Michael A. Fiorillo, and Paul G. Davidson* 289
Gynandroblastoma in Pregnancy: Case Report and Review of Literature *Tamara Kalir, and Frederick Friedman, Jr* 292

SPECIAL ARTICLE and EDITORIAL

Impact of the Libby Zion Case on Graduate Medical Education in Internal Medicine *Jeffrey M. Brensilver, Lawrence Smith, and Christopher S. Lyttle* 296
The Libby Zion Case Revisited: What Have We Learned? *Barry Stimmel* 301
Reply by Authors 303

STUDENT CORNER

Unexpected Factors Predict Control of Hypertension in a Hospital-Based Homeless Clinic *Josiah Child, Michael Bierer, and Kim Eagle* 304

DERMATOLOGY NOTES

Generalized Morphea *Marsha Gordon, and Mark Lebwohl* 308

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Money, Medicine, and Methuselah

Christine K. Cassel, M.D.

Everywhere you look, the message is the same: Americans are getting older. The dramatic increase in the number of Americans aged 65 and older reflects a success story. It is a miracle of modern medicine that many people can expect to live well into their eighties, nineties, and even beyond. Three score and ten is no longer viewed as the "natural" time to die. Today, it is not unusual to hear of someone in his mid-70s who has survived pneumonia and lived long enough to have a successful coronary bypass operation, hip and knee replacement, and cataract surgery, successful procedures to keep that person functional until well into, or even past, his nineties. Clearly, we should be elated at the unprecedented gains in life expectancy that we have experienced over this past century. But are we prepared to face the enormous scientific, clinical, and social challenges that lie ahead as a consequence of this historical phenomenon?

Until not too long ago, high fertility and mortality rates kept our country young. During the Civil War years, half the population was under age 20 and most people were not expected to live to age 65. Since 1900, however, the number of persons 65 and over has increased 11-fold, while the population under age 65 has merely tripled. Between 1965 and 1995 alone, this older population grew by 82%, and between 1980 and 1995 by 28%, reaching a historical high of 33.5 million people. Today, approximately 1 in 8 Americans is over age 65, and by the year 2030, this could increase to about 1 in 5 (1) (Fig. 1).

The oldest old, our 85-plus population, now the most rapidly growing segment of the total population, has witnessed the most dramatic change. In 1994, 3.5 million people were in this age group, compared to only 122,000 in 1900. Although this group constitutes only 1% of our country's total population, it is having a major impact on our country's health care and social delivery systems, as well as on our family structures. In subsequent decades, we can expect to see even more dramatic increases in the number of people in this category—almost 9 million in 2030 and 19 million by 2050 (1).

The number of centenarians in the United States is also growing (Fig. 2). According to one estimation method, the number doubled between 1980 and 1990, and the most recent available data report that while those born in 1879 had only a 1 in 400 chance of living to age 100, those born in 1980 have increased odds of 1 in 87 (2).

Life expectancy (the average expected years of life remaining for an individual at a certain age) is based on observed death rates for a population in any given year. Even by 1900, life expectancy was only 47 years. But times have changed; today life expectancy for women in the United States is 79 years; for men it is 72 years, more than a 28-year increase since the turn of the century (3). In 1965, the year Medicare was created, policy makers assumed life expectancy had plateaued at 70 years; three years later, however, mortality rates for older people declined again and have continued to decline, much to the amazement and consternation of many people, including the Medicare Trustees and the Social Security Administration.

Americans are growing older, but to forecast the social and health policy implications, we need to ask ourselves why more people are surviving to

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Address correspondence to: Christine K. Cassel, M.D., Professor and Chairman, The Henry L. Schwartz Department of Geriatrics and Adult Development, Box 1070, One East 100th Street, Mount Sinai School of Medicine, New York, NY 10029

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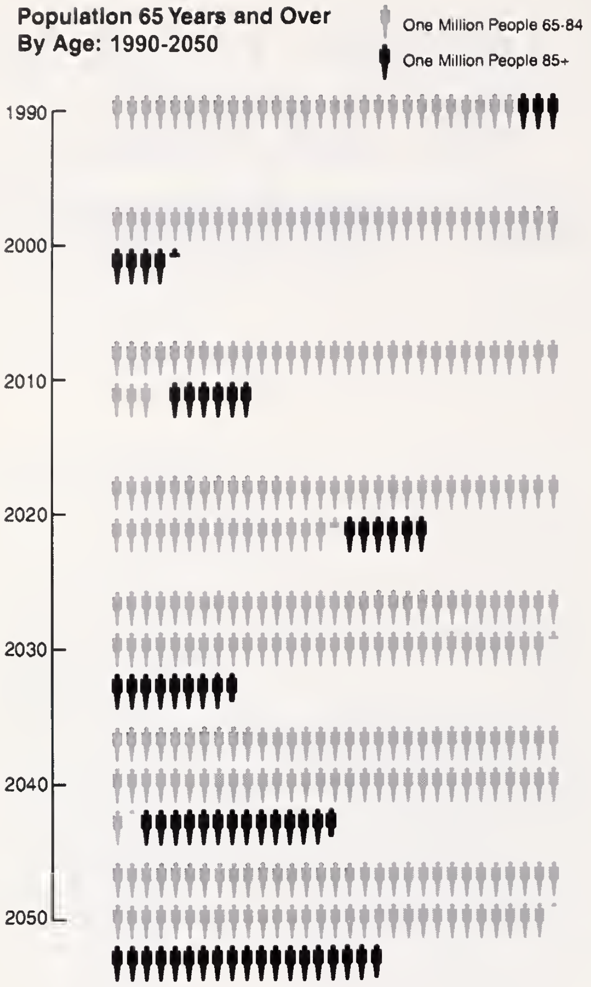

Fig. 1. Growth of the 65+ population, by age group: 1900 to 2050. *Source:* U.S. Bureau of the Census. Sixty-Five Plus in America, P23-178RV; Population Projections in the United States, by Age, Sex, Race, and Hispanic Origin: 1993 to 2050. P25-1104.

what is considered the natural period, possibly even the genetic limit, of life. During this century, the causes of death have shifted dramatically. In the early part of this century, infectious and parasitic diseases were the chief causes of death. Until the mid-1960s, social conditions, not medical care, accounted for most of our gains in life expectancy. For example, from 1860 to 1960, the death rate from tuberculosis steadily declined, not because of antibiotics but because of improved overall living and working conditions; it was not until 1945 that antibiotics were introduced that actually could kill the tubercle bacillus. Since then, public health and sanitation conditions, nutrition, and general medicine, antibiotics, and acute care interventions have improved, and Americans have been making significant lifestyle changes. We can also look forward to potential antioxidant, hormonal and drug, and gene therapies.

The gains we have made in preventing, delaying, and treating the non-fatal but disabling diseases of old age are less striking. Since 1967, causes of death have been predominantly diseases related to old age: degenerative cardiovascular diseases, stroke, and cancer (Fig. 3). Yet even though we have been making marked gains in life expectancy, these primary causes of death have remained the same for the past 30 years. Improved lifestyles and medical treatments have delayed the onset of most of these fatal diseases but have not really "prevented" them, with the single major exception of tobacco-related emphysema and lung cancer when smoking is stopped. But heart disease is multifactorial, and a combination of behavioral and medical factors have delayed onset of heart disease, not prevented it. It may no longer be common for a man to suffer his first heart attack in his forties, but it is not at all uncommon for it to happen when he is in his seventies or even eighties.

We may have succeeded in postponing when these diseases kill, but sometimes the consequence is longer life with chronic illness (4); in other words, we have so successfully treated these fatal diseases that we have transformed them into chronic illnesses. We save the lives of many who would have died from heart attacks, only to have them survive to be vulnerable to other chronic illness or to live with chronic heart diseases such as congestive heart failure. The challenge of medicine is no longer primarily diagnostic; rather it is management of chronic illness. The shift of acute rapidly fatal illness to chronic illness is accompanied by more years of life during which people are also vulnerable to chronic disabling and nonfatal illness. Living longer means that we have a greater chance of suffering disability from chronic musculoskeletal diseases, such as osteoarthritis and osteoporosis; degenerative neurological diseases, such as Alzheimer's and Parkinson's diseases; and sensory impairments, such as hearing loss and blindness. Most of these are not viewed as potentially fatal, and yet they may have devastating consequences. Half of our over-65 population suffer from arthritis, 36% from high blood pressure, 33% from heart disease, 29% from hearing impairment, 17% from cataracts, and 10% from diabetes (5). The probability of having multiple chronic illnesses also increases with age: 88% of those 65 and older have at least one chronic condition to manage, and 69% have more than one (6), and comorbidity rates are especially high for older women (7). Although many of these conditions are not life threatening, they do lead to disability and consequently affect quality

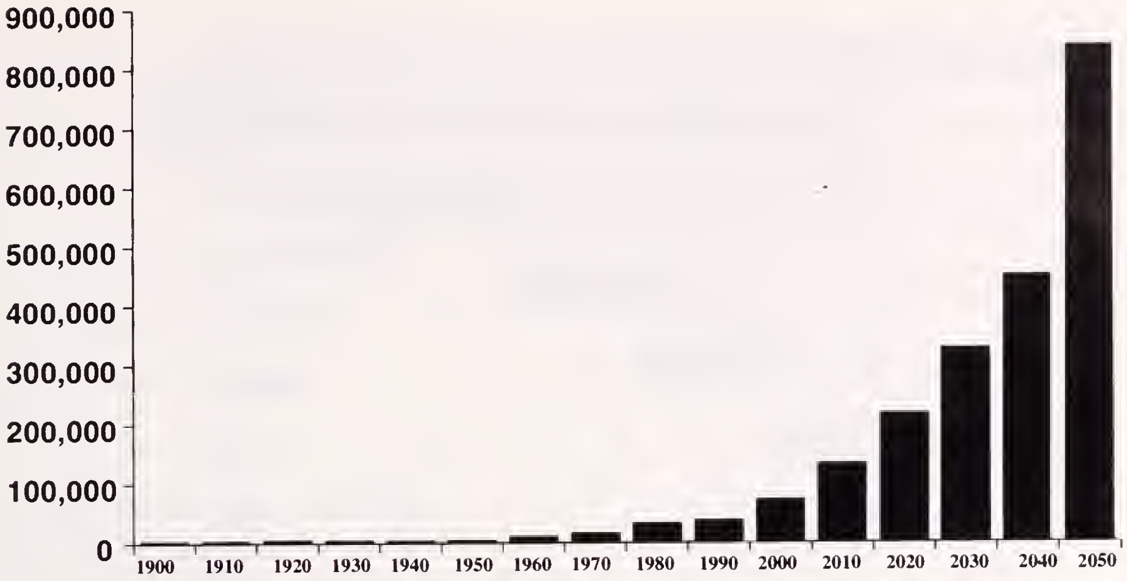

Fig. 2. Number of centenarians in the United States for the period 1900 to 2050. *Source:* Modified from Spencer G, Goldstein A, Taeuber C. U.S. Bureau of the Census, America's centenarians: Data from the 1980 census. Washington, DC: US Bureau of the Census; Current Population Reports, Series P-23, No. 153; 1987; and from information obtained from telephone contact, U.S. Census Bureau, Interagency Forum on Aging-Related Statistics.

of life. Age strongly correlates with the prevalence of disability (Fig. 4); although the overall disability rate in the United States is 20.6%, it is 47.3% for persons aged 65 to 79 and 71.5% for those aged 80 and older (7).

In studying changes in disability with increasing age, we can divide life expectancy into independent and dependent stages. Researchers have been attempting to predict active life expectancy, that segment of life during which a person can

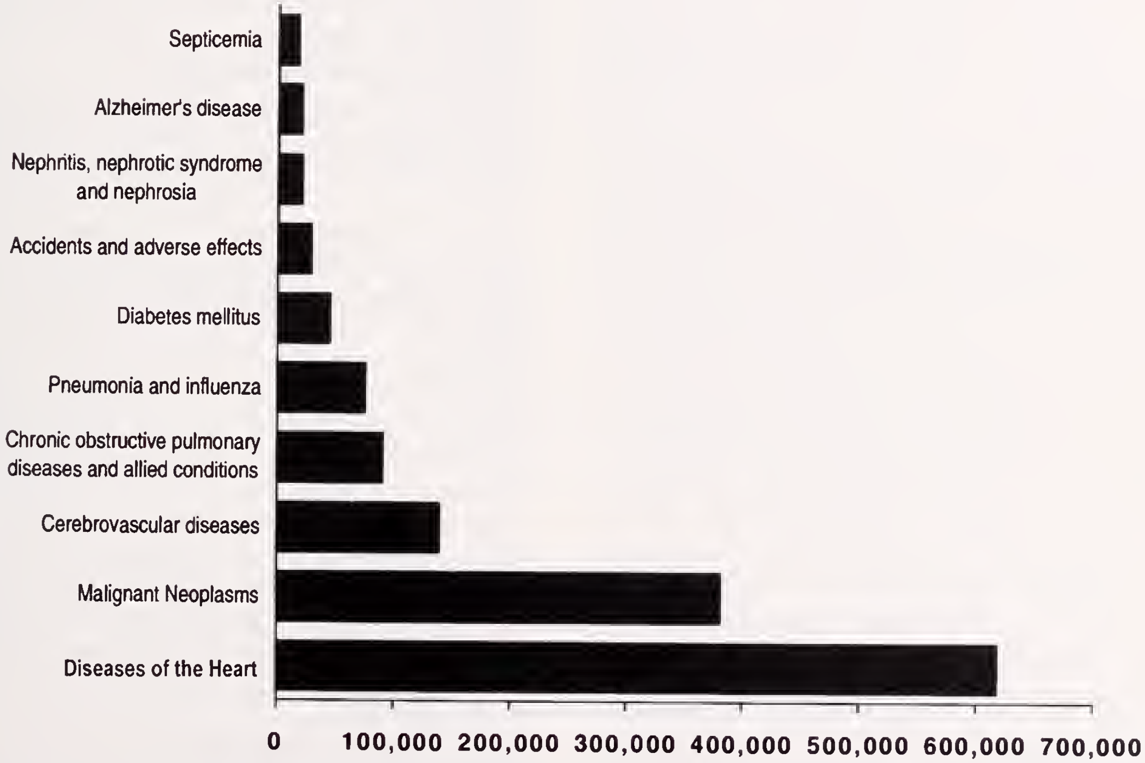

Fig. 3. Leading causes of death for population ages 65+ years for 1996. *Source:* Monthly Vital Statistics Report. Washington, DC. Dept. of Health and Human Services, Center for Disease Control. Vol 45 No. 3 (5)2, Oct. 4, 1996.

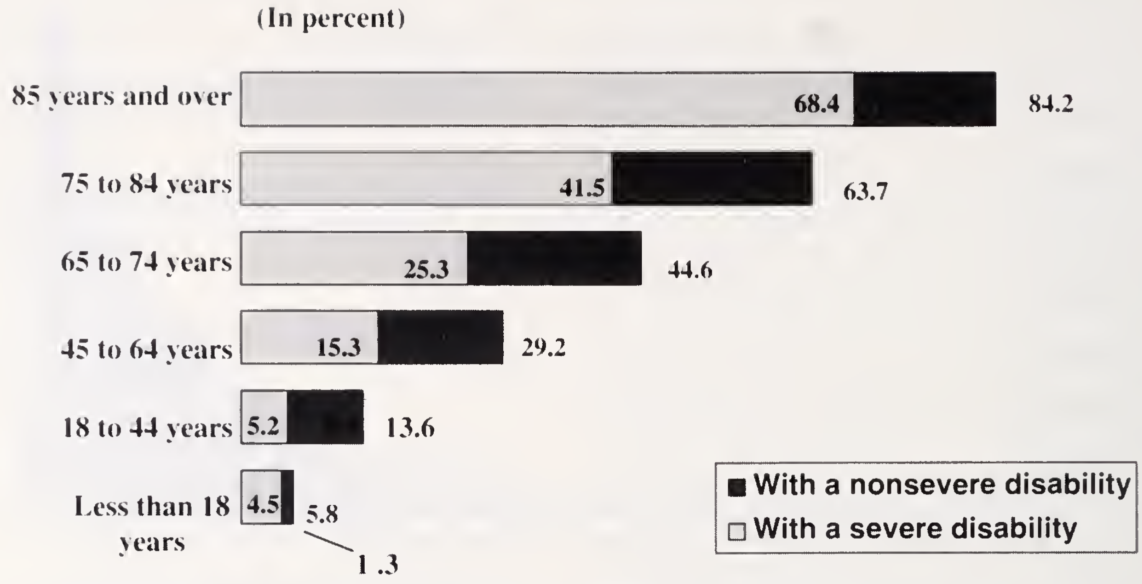

Fig. 4. Disability prevalence by age: 1991 – 1992. Percent with specified level of disability. *Source:* Reference 7.

function independently. Some have suggested that for every year of increased life expectancy, 9.6 months (80%) will be spent disabled (8). That means that whereas today, on average, a 65-year-old can expect to live another 17 years, approximately 6 of those years will be lived in a disabled state, and an 85-year-old will spend almost two-thirds of his remaining years with some type of severe disability. Our health care delivery system, substantially built on an acute care construct, often fails to meet the needs of those suffering from chronic conditions, especially nonfatal conditions (6).

In an aging population with a high prevalence of chronic illness, functional limitation, rather than disease status, is a more useful indicator of the need for health and social services. Even though some people remain independent throughout life, dependency rates increase rapidly with age. Although 11.5% of those aged 65 to 79 who are living in the community report that they need the help of another person with activities of daily living (ADLs) or instrumental activities of daily living (IADLs), for those aged 80 and older, this percentage jumps to 34.1 (7). [ADLs include bathing, dressing, transferring, toileting, continence, and eating. IADLs include handling personal finances, preparing meals, shopping, traveling, doing housework, using the telephone, and taking medications.] Functional limitation strongly correlates with nursing home admission: only slightly more than 3% of elderly community residents report being limited in 3 or more ADLs, compared with 69% of those living in a nursing home (9).

A recent report for the Administration on Aging states that all available data suggest the number of persons living in the community who need assistance with ADLs, as well as those who will become severely disabled, will increase sharply between 1995 and 2040 (10). The outcome of this increase will be higher medical spending, since we spend five times more for persons who are limited in activity due to two or more chronic conditions than we spend for those who have no activity limitations (11).

Life expectancy may have risen, but the onset of these disabling, aging-dependent diseases (diseases that increase steadily in frequency with age) has remained the same. Running marathons, maintaining the best diet, quitting smoking, managing stress, and other lifestyle changes will not alter the onset of any of these disorders. If older Americans' susceptibility to disabling disease and aging conditions could be appreciably delayed, they might live healthier, longer lives and avoid losing their independence, and our nation would experience vast social and economic benefits, as well. The Alliance for Aging Research estimates that for each month that we can postpone physical dependency for older persons, our country would save $5 billion, at minimum, in health and long-term care costs; a five-year delay of the onset of chronic conditions would have enormous social and economic benefits (12) (Fig. 5).

We need to develop medications, devices, and environments for the millions of elderly who are affected so that they can have optimal quality of

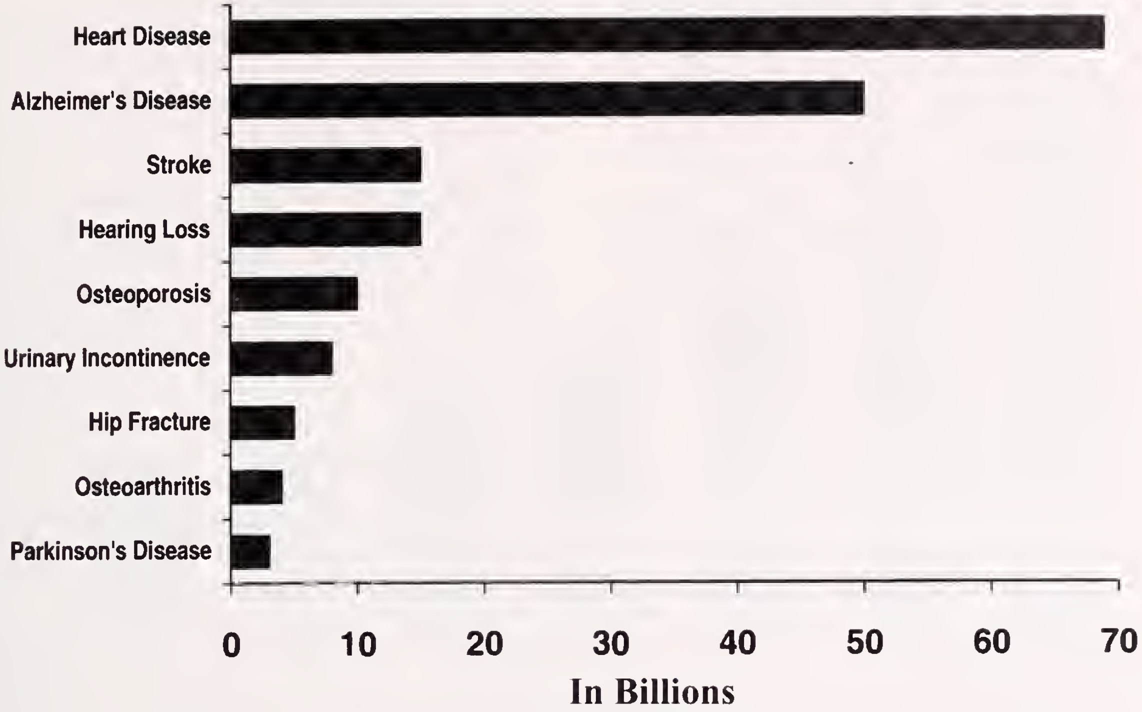

Fig. 5. Annual cost savings in U.S. dollars if onset were delayed by 5 years. *Source:* Modified from Reference 12

life. Without major breakthroughs in scientific research, these chronic disabling conditions will continue to occur and have serious implications. These are research areas that may not be as glamorous as discovering the answer to heart disease or cancer, but these are among the most important challenges facing medicine; we must concentrate our scientific efforts on these diseases in order to brighten the outlook for our "prolonged" life expectancy.

Postponing the precipitous rise in hip fractures, a major public health problem today, by just six years would reduce the number of hip fractures by 50% and substantially reduce disability and health care costs for the older population (13). In addition to the human costs of high mortality and suffering, the economic costs are also significant. With costs running near $40,000 per fracture, it costs nearly $10 billion per year in medical care and lost income. If the prediction of the American Academy of Orthopedic Surgeons is correct, given the aging of our population, by the year 2020, the cost of hip fractures will double, and by 2050, the number of today's (nearly 350,000) hip fractures a year will double (12).

Dementia, although by itself not a fatal disease, causes severe dependency and disability. Approximately 4 million people in the United States today have Alzheimer's disease, a major cause of dementia, but this is only a rough estimate. Because it is not always reported on death certificates, prevalence estimates are based on surveys from different communities whose findings vary. Most report that 25 to 35% of those aged 85 and older have some kind of dementia, including Alzheimer's (14). One study reports that Alzheimer's alone strikes more than 47% of those over age 85 (15); another estimates that it costs the nation more than $100 billion each year (16), and this does not account for the non-financial costs to families and communities who bear the personal burden of the care required for people with dementia.

A recent study reports that chronic disability is declining among the elderly (17); however, with declining mortality rates, medical advances, and improved life-styles and medical care, the number of people 65 and older will continue to account for a larger percentage of our overall population, and subsequently the number suffering from chronic conditions will require medical and long-term care that, in turn, will have a dramatic impact on health care use and costs.

Now that there are so many opportunities for treatment and prevention of the major disabling diseases of old age, it is all the more important that physicians understand the basic causes of aging and how to manage patients with multiple and complex problems. Geriatricians are trained to evaluate the patient comprehensively and advise the patient and family on a course of treatment that will enhance function and promote independence as long as possible. Although many other medical specialties measure their success in terms of survival rates or the working of a specific body organ, the geriatrician measures success in terms of a patient's function. To make the full resources of this whole-patient approach available, geriatricians also work closely with other health professionals, especially nurses and social workers, as an interdisciplinary team in providing continuity of care.

Yet fewer than 1% of our country's physicians are certified geriatricians, trained to handle the complex medical needs of the elderly. The Alliance for Aging Research estimates that we currently need 20,000 physicians with geriatric training, 13,000 more than are currently available; it estimates that by 2030, we will need nearly 37,000 geriatric-trained physicians, but if the present trend continues, only 8,880 will be available (18). In addition, according to the American Geriatrics Society, by the year 2000, many clinical and academic geriatricians will retire (19). Not all older people need a geriatric specialist, but primary care physicians treating the elderly should be trained in geriatrics. Yet, a relatively small proportion of the 7,000 internists and family physicians currently certified as having added qualifications in geriatric medicine have received formal geriatric training, and the current requirement for completion of an accredited two-year geriatric program has reduced the number eligible for certification to about 100 per year. Our medical schools face a severe shortage of academic geriatricians to teach undergraduate students and residents. The Institute of Medicine projects that to establish high-quality training in geriatrics in all of our medical schools by the year 2000 would require at least 3 to 5 academic geriatricians in every major teaching center, but to provide the needed contributions in geriatric research and teaching, we must double the number of faculty who graduate each year from geriatrics fellowship programs (20, 21).

Young people are not rushing to enter the field of geriatrics. Persisting negative attitudes about aging in our society prevent many of the best and the brightest from realizing how the aging of the population represents a frontier for American medicine, including scientific mysteries to be solved, innovations in health care to be invented, and the human dimension of caring for grateful patients and families who really benefit from thoughtful, competent, meticulous geriatric care. However, we continue to train our nursing and medical students and our interns in the hospital setting rather than where we treat most of our elderly population, in nursing homes, in hospices, and in their own homes.

Today, the United States has nearly twice the number of nursing home beds as hospital beds at a time when more and more hospitals are closing their doors. Hospitals increasingly are limited to intensive care or major surgery, and much of the real continuity management occurs outside the hospital. Many disorders previously requiring inpatient care are now often treated on an outpatient basis. And, for every person in a nursing home bed, there are two to three times as many people in the community, especially within the 85-plus population, with equal levels of disability who are being cared for by a combination of formal and informal services. Home care may be preferred by most patients, but it is not inexpensive. And it is primarily women who perform the home care, posing an intriguing policy challenge. As a society, we have to decide whether we want women to quit their jobs to take care of their aging parents and grandparents, or whether we want to build a network of professionally delivered services so that women can stay in the job market and contribute to the economy.

Given the enormous growth in the home care industry in its efforts to control costs and the preference of most people to recover from illnesses at home rather than in a nursing home or hospital, utilization rates for nursing homes are no longer keeping pace with the increase in the overall elderly population. Although the number of nursing home beds increased by 9% between 1985 and 1995, the number of nursing home residents increased by only 4% (22), given the availability of intermediate care (that between complete independence in the home and dependence in a nursing home). This decrease in nursing home admissions, however, is not true for the old-old; approximately 25% of those 85 or older reside in nursing homes, and the proportion of nursing home residents age 85 and older doubled between 1960 and 1990 (Fig. 6).

Despite the increase in intermediate levels of care being provided in the home by relatives and friends, and in small group settings, those patients with higher impairment levels will continue to need labor-intensive, sophisticated 24-hour skilled nursing home supervision into the indefinite future, unless, among other things, medical technology can increase active life expectancy among the oldest old.

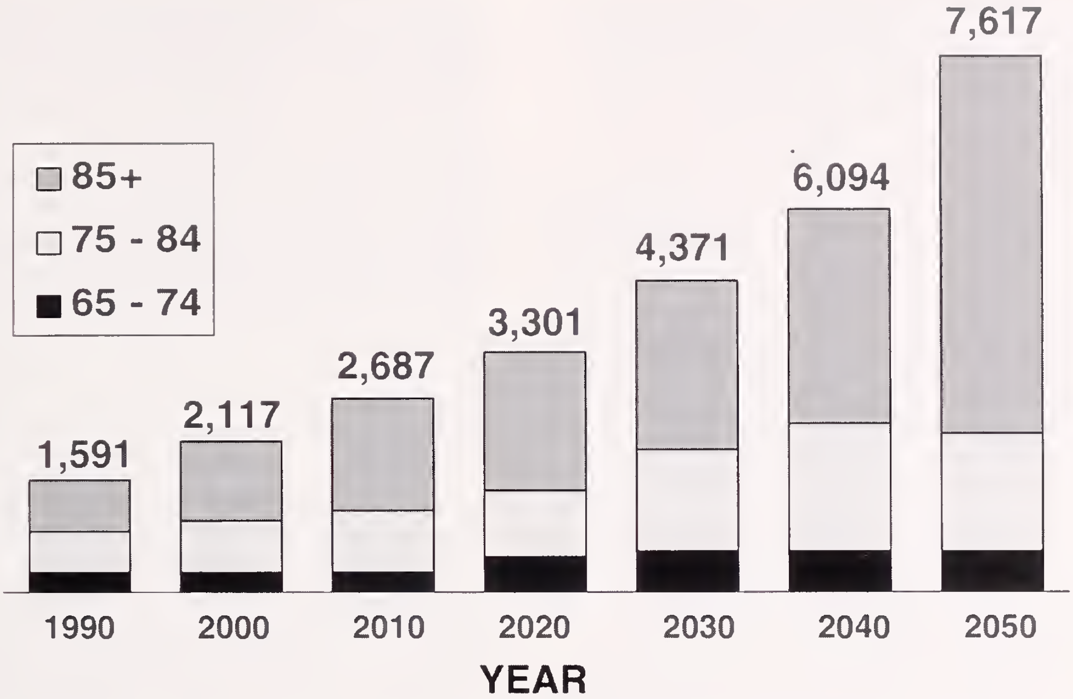

Fig. 6. Projections of the nursing home population aged 65 years and older by age group; 1990–2050. Population given in thousands. *Source:* Based on nursing home utilization rates from the 1990 census (press release CB93-117) and projections of the population by age (Current Population Reports, P25-1104).

A team of researchers from the University of Illinois and the University of Chicago predict that if residency ratios remain unchanged, the number of those residing in nursing homes will double or triple by 2030 and for those aged 85 and over, the number could rise past 300%. Even if residency ratios decrease by 6.5% each decade, they report that the number of nursing home residents will increase at least by 57%. The researchers also project substantial growth, with increasing age, in the number and proportion of older people in nursing homes, as well as significant increases in the number and proportion of older disabled individuals being transferred from home health care to nursing home care; also expected are significantly larger percentage increases in nursing home care as compared to home care, particularly at ages 85 and older as compared to ages 65 and older (23).

Projected costs for long-term care dwarf even those projected for hospital physician services. Cost prevented any serious discussion of long-term care during the 1992–93 Health Reform Debate, and long-term care continues to be avoided by policy makers. Assuming no significant changes in public policy, nursing home and home care costs are projected to increase an average of almost 10% annually compared with an average of less than 8% for other personal health expenditures. In 1994, $733 billion was spent on nursing home and home care; cost projections for 2004 are more than double the 1994 figure, at $1508 billion. Between 1996 and 2004, home care spending is projected to rise even faster than nursing home spending, from $36 billion to $95 billion for home care as compared to $88 billion to $169 billion for nursing home care (9).

With no really affordable and useful long-term care insurance, how do we pay for long-term care? One-third comes from out-of-pocket (meaning families as well as older people themselves), with the remainder from Medicaid (38%), Medicare (16%), private insurance (6%), and 7% from other sources (24). Although the proportion is slowly growing, only about 4 to 5% of the elderly currently have long-term care insurance (25). On its current course, this fragile "house of cards" will ultimately collapse.

The money to pay for the projected long-term care cost increases cannot come only from the elderly. Ironically, unlike when Medicare was first developed, they are no longer viewed as a sympathetic and deserving population, but rather as those "rich old geezers" who are causing all these problems for the younger population. Yet in 1994, the median income of households maintained by persons aged 65 and over was only $18,095 as compared to $32,264 for younger households (26). There is a pronounced economic disparity among the subgroups within the elderly population; women, Blacks, persons living alone, the old-old, those living in rural areas, and particularly those with a combination of these attributes are more likely to live in poverty (26).

As a society we also must gain acceptance of death as a natural part of life. Physicians must learn to recognize that there is a time when the focus must shift away from curing a patient towards assuring the person that his or her final days will be lived in comfort and dignity. Finding that too many dying patients are suffering preventable pain and distress due to inadequate care, a recent Institute of Medicine report has called for changes to improve end-of-life care, including better training of health care professionals (27). People who are dying should be able to count on skillful and compassionate care that reflects their own wishes and those of their families. Yet various studies reflect that physicians often do not know or understand what patients and their families want and have difficulty recognizing pain and other symptoms because their training focuses more on acute illness and heroic rescues rather than on assessing and managing pain or helping those with terminal illness prepare for death.

If Americans think about death at all, the ideal is to die suddenly from a heart attack, without any suffering and without any burden on their family and the other people who care about them. And it is the prevention of these kinds of deaths to which Congress pays the most attention and which receives most of society's investments. If we would just invest that identical amount of money in other quality-of-life areas, we could improve the outcome for people who are going to live longer lives and even capitalize on the success of this increasing life expectancy.

Since it is no longer so unusual to hear of someone celebrating his or her 100th birthday, we must begin to contemplate what the prospect of living that long means for ourselves and for our society. The "burden perspective" argues that all of this aging is a terrible weight for our society to bear. A contrary view is that this is not a burden, but a tremendous success, because civilization itself brought about the first part of this century's increasing life expectancy and because the success of health care and medicine brought about the second phase of increased life. The challenge we must face is that we must determine how to make society perceive it as the success that it is. One solution is to increase our capability to look at health care from a perspective that makes sense to people suffering from chronic illness.

Ironically, the answer may lie in managed care. Some versions of managed care offer real promise for the way in which we actually might fulfill the needs of an aging society by promoting comprehensive, functionally oriented approaches to chronic illness. The key factor is coordination of care, which is often lost in our fragmented fee-for-service system. The transition to managed care has placed more emphasis on the important role of training primary care physicians in order to provide them with the skills necessary to treat the growing numbers of elderly patients. In their attempt to provide cost-effective, quality health care services, their focus has turned to primary care to promote health, prevent illness, and diagnose the early onset of disease. Full risk capitation allows flexibility of resource use and could allow a greater meshing of acute and long-term care. If we could constrain the acute care costs, we could also expand other benefits, such as providing hearing aids and glasses. That would dramatically improve the quality of life of older people—benefits that traditionally have never been covered by any program. We could emphasize preventing decline in health as well as illness, both in hospitals and in the community.

Managed care has many risks as well, especially if financed inadequately. Other concerns include inadequate provider expertise, burdens on family as our enthusiasm about home care causes us to have patients return home too soon, and delay of needed care because attempts to ration services may withhold useful interventions. There are many promising technological advances available to improve function in old age. Physicians should not believe that all geriatrics is fatalistic or custodial care. This is where the excitement is and where the expertise is needed—to know when aggressive interventions are warranted and when they are not. And looking at the entire picture under capitation, we see an immense opportunity for long-term care to finally obtain its rightful place in trying to provide a true and beneficial array of services.

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New Concepts in the Pathogenesis and Treatment of Allergic Asthma

Mitchell H. Grayson, M.D., and Bruce S. Bochner, M.D.

Abstract

This review delineates the currently accepted, multifaceted approach to the care of an asthmatic patient. Therapy is based on our improved understanding of the pathophysiology of this complex disease, in which IgE and mediators derived from many cells contribute to airway inflammation. The latest trends in therapy involve the institution of anti-inflammatory medications relatively early in disease. With the recent availability of a novel class of therapies, the leukotriene antagonists, and other therapies based on antagonism of adhesion molecules, cytokines, chemokines, or IgE itself, the future may provide additional alternatives for the physician treating patients with asthma. **Key Words:** Asthma, eosinophil, adhesion, apoptosis, cytokines, chemokines, education, IgE, immunotherapy, treatment.

Introduction

ASTHMA IS A HETEROGENEOUS DISORDER of the airways characterized by airway inflammation, hyperresponsiveness, and obstruction. The disease may occur as a result of both allergic and nonallergic mechanisms. Despite improved therapies, it remains a common disease that continues to have a great impact on our health care system. For example, in the United States alone, nearly 10 million people are affected, with more than 6 billion dollars spent in 1990 on asthma-related health care (1). Perhaps more important than the sheer numbers and financial issues is the fact that in the period 1980–1987 the prevalence of asthma in the general population actually increased by 29%. Not only are there more patients with the disease, but they appear to have more severe asthma, because the mortality rate over the same period increased by nearly a third. Therefore, it is crucial that we continue to enhance our knowledge of this disease and strive to expand our therapeutic options. This review will describe some of the implications of recent research on the pathophysiology of asthma and the rationale for treatment of this complex disease.

Pathophysiology

Clinically, asthma can manifest itself as cough, wheezing, shortness of breath, chest tightness, and/or increased bronchorrhea (2). Most of these signs and symptoms are due to bronchial hyperreactivity and reversible airways obstruction. In an acute asthmatic attack, edema, increased mucus production, and smooth muscle contraction (bronchoconstriction) result from release of mast-cell-derived mediators, such as histamine. With time, a more prolonged bronchoconstriction can occur as numerous eosinophils and subsets of T lymphocytes (the Th2 subset of helper T cells) accumulate

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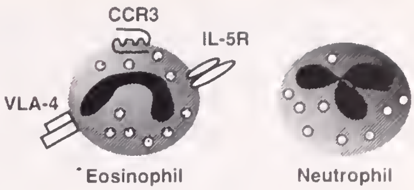
From the Department of Medicine, Division of Clinical Immunology, The Johns Hopkins University School of Medicine, Johns Hopkins Asthma and Allergy Center, Baltimore, MD. Address correspondence to Dr. Bruce S. Bochner, Johns Hopkins Asthma and Allergy Center, Room 3A.62, 5501 Hopkins Bayview Circle, Baltimore, MD 21224-6801.

in the airway mucosa, with subsequent thickening of the basement membrane, airway epithelial desquamation, and subepithelial fibrosis. These changes are thought to be related to the release of substances from the eosinophil, including leukotrienes (LT, especially LTC_4), major basic protein (MBP), and eosinophilic cationic protein (ECP) (3). The selective accumulation of eosinophils can be easily demonstrated by bronchoscopy. In a normal, non-asthmatic subject, there are <1% eosinophils in bronchoalveolar lavage (BAL) fluid. However, in an asthmatic, the number of eosinophils may approach 5%, and within hours of endoscopic challenge with allergen, eosinophils can account for 50–70% of cells obtained by BAL (4). Thus, the ability of the lung to generate proinflammatory signals for selective eosinophil recruitment as a result of IgE-dependent activation of resident tissue cells is quite striking. How the eosinophil is preferentially attracted to the airway mucosa is an area of intense research. Several mechanisms have recently been proposed (Fig. 1) and are discussed below.

Mechanisms of Selective Cell Recruitment to the Airways in Asthma

Adhesion Molecules

In order for leukocytes to leave the circulation, they must first bind to the luminal surface of the vascular endothelium. Cytokines can activate endothelium and lead to the expression of adhesion molecules. Although endothelial cells are capable of expressing several different types of adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), E-selectin, and vascular cell adhesion molecule-1 (VCAM-1), it is the latter molecule, VCAM-1, that has received particular attention in allergic inflammation (5). This molecule may impart specificity to the cellular infiltrate seen in asthma because eosinophils possess on their surface the integrins α4b1 (VLA-4) and α4b7 which are the counter ligands for VCAM-1. Neutrophils, in contrast, do not possess these surface integrins. Two cytokines, IL-4 and IL-13, are unique in that they selectively induce expression of VCAM-1 without affecting other endothelial adhesion molecules (6, 7). This is of particular interest because these same two cytokines are required for the production of IgE (8). In symptomatic asthmatics, increased VCAM-1 levels in the lung have been detected by immunohistochemistry and other methods. Furthermore, the use of α4 integrin blocking antibodies markedly decreased the influx of eosinophils into the airways in animal models of asthma (9, 10).



Binds to VCAM-1 on endothelium via the adhesion molecule VLA-4	Yes	No
Exhibits chemotaxis to C-C chemokines such as RANTES, MCP-4, and eotaxin via the CCR3 chemokine receptor	Yes	No
Displays enhanced biological activity and prolonged survival in the presence of IL-5 via the IL-5 receptor	Yes	No

Fig. 1. Examples of possible mechanisms responsible for selective eosinophil accumulation.

Chemokines

Another mechanism may involve eosinophil-selective chemotactic factors. Chemokines, a family of small chemotactic cytokines (8–10 kD) with conserved patterns of cysteine residues, have been discovered recently. These molecules can be further divided into subfamilies (such as the C-X-C and C-C families) based on whether there is an intervening amino acid between the first cysteine pair (11). The C-X-C chemokines, of which more than 10 are currently known, are generally chemotactic for neutrophils (e.g., IL-8), while the C-C chemokines, of which more than 20 are currently known, are chemotactic for mononuclear cells, eosinophils and basophils. Certain C-C chemokines, such as RANTES, eotaxin, and MCP-4, are especially active on eosinophils; among these, the activity of eotaxin is most specific for eosinophils. This specificity resides in the type of chemokine receptor that the cell expresses. For example, normal neutrophils (but not eosinophils) express CXCR-1 (the type 1 C-X-C receptor) that binds several C-X-C chemokines (such as IL-8), while eosinophils (but not neutrophils) express CCR-3 (the type 3 C-C receptor) that binds several C-C chemokines such as RANTES, eotaxin, and MCP-4 (12). These latter chemokines are both potent and selective chemoattractants for eosinophils, and their presence in the upper and lower airways, especially in the epithelium where they are produced in significant quantities, has now been found in allergic airways inflammation (13–15). The ability of chemokines to cause selective cell accumulation in animals and humans *in vivo* has been demonstrated in studies with intradermal injections of IL-8 or RANTES. In studies with IL-8, neutrophils rapidly accumulated at the site, while in the RANTES studies, large numbers of eosinophils were seen, often within 30 minutes (16, 17). Thus, the ability of certain chemokines to cause accumulation of particular types of leukocytes suggests that these molecules, especially those of the C-C subfamily, may be important in allergic inflammation. Efforts by pharmaceutical companies to generate new therapies that target the epithelial production of chemokines, or the chemokine receptors themselves (e.g., CCR-3 on eosinophils), are already underway.

Cytokines and Apoptosis

A third mechanism may involve eosinophil-selective cytokines for hematopoiesis, activation and survival. Three cytokines, IL-3, IL-5, and GM-CSF, share these properties; among these, IL-5 appears to be the most specific and important (18). These cytokines induce *in vitro* conversion of eosinophils to the hypodense (activated) phenotype and increase production of leukotrienes. Antibody-mediated cellular cytotoxicity also is augmented (19). The major sources of IL-3 and IL-5 appear to be T lymphocytes. GM-CSF is produced by many different cell types, including airway epithelial cells (20). The efflux of eosinophils into asthmatic BAL fluid may therefore be due to cytokine and chemokine production by the epithelium. In allergic asthmatics, these cytokines probably help to maintain peripheral blood eosinophilia, enhance eosinophil adhesion, and potentiate their responsiveness to C-C chemokines (21).

So far, several mechanisms by which the eosinophil can gain entry into the bronchial mucosa and become activated have been summarized. However, it is clear that there must be a system in place to limit the number of cells so that the reaction can be down-regulated after the initial stimulus has been removed. Apoptosis, which is akin to cellular suicide, may be the mechanism by which the number of eosinophils in the lung is limited. This phenomenon, which differs from cell damage and necrosis, involves a process termed *programmed cell death* and is characterized by cell shrinkage, untethering of the plasma membrane, condensation of chromatin, and DNA degradation by activation of DNases within the cell nucleus (22). Cells undergoing apoptosis become susceptible to phagocytosis by macrophages, because they display new adhesion molecules on their surface, and therefore can be readily engulfed before their contents are released via necrosis (23). Therefore, apoptosis may represent the body's way of turning off an inflammatory response once the initial trigger has been removed. With respect to allergic diseases, one way in which to induce eosinophil apoptosis is to withdraw or neutralize the needed survival-promoting factors; this is the rationale behind the development of an anti-IL-5 antibody (24). The hope is that this medication will decrease the number and survival of eosinophils in the periphery, thus leading to less inflammation in asthma. Another approach is to directly stimulate the cell to undergo apoptosis. As an example of this mechanism, for eosinophils and other cells, the process of apoptosis can be initiated by crosslinking the cell surface protein fas (25). Studies performed *in vitro* with human eosinophils, or *in vivo* in a mouse model of eosinophilic airways inflammation, revealed that anti-fas antibody can cause eosinophils to undergo apoptosis within hours (26).

Recently, a factor was discovered in BAL fluids of asthmatics that caused eosinophil apoptosis. This factor turned out to be the lidocaine used to anesthetize the airway for bronchoscopy (27). Even though this accidental discovery seemed trivial, one early study of the use of nebulized lidocaine for asthma appears promising. In an open trial, Hunt et al. (28) treated 20 oral steroid-dependent asthmatics with nebulized lidocaine (40–160 mg four times daily) for up to 24 months and monitored steroid dose requirements. Oral corticosteroids were successfully discontinued in 13 of the patients. Four patients were able to reduce, but not eliminate, their oral steroid use. The major side effects of the lidocaine were bitter taste, numbness of the pharynx, and regurgitation. Although the motivation for the study involved the potential anti-eosinophil effects of lidocaine, the mechanism for the apparent clinical benefit in this study was not investigated. This small, uncontrolled study suggests that nebulized lidocaine may have a potential role as a steroid-sparing agent in asthma; another controlled study is already underway. Further studies are needed before this therapy can be readily embraced.

Clinical Considerations: New Insights

The goals of asthma management are numerous: control or prevention of symptoms, reduction of exacerbations, and maintenance of normal or near-normal pulmonary function. Attainment of these goals will lead to the asthmatic being able to maintain a normal level of activity and exercise. Additionally, optimal asthma management should prevent irreversible airway obstruction and subsequent asthma mortality, while avoiding adverse effects from medications (2).

Asthma Severity Classification Schemes

In 1991, the National Heart, Lung, and Blood Institute of the National Institutes of Health and other international organizations, developed a stratified asthma disease severity classification system to aid in appropriate management (29, 30)[1]. Asthma severity, based on this system, can be classified into either mild intermittent, mild persistent, moderate persistent, or severe persistent. The classification depends upon four criteria: weekly symptoms, monthly nocturnal asthma, peak expiratory flow rates (PEFR), and PEFR variability. Mild intermittent asthma is characterized by intermittent symptoms (<2 episodes/week) with infrequent nocturnal symptoms (≤2 nights/month) and short exacerbations with normal PEFR between attacks. PEFR in these patients are ≥80% of their baseline with little (<20%) variability in their measurements. Mild persistent asthma is similar; however, these patients have symptoms more than twice weekly, but not daily. Nocturnal symptoms are more frequent (>2 nights/month). These patients have PEFR ≥80% of baseline with 20–30% variability. The moderate persistent asthma patient has ≥2 episodes per week of symptoms with frequent nocturnal complaints (>1 night/week). Their PEFRs are 60–80% of baseline with >30% variability. They have daily symptoms requiring daily use of a bronchodilator. Severe persistent asthmatics are characterized by continuous, limiting symptoms with frequent nocturnal asthma. Additionally, these patients have highly variable PEFR measurements (>30% variability) that are ≤60% of their baseline. These criteria are useful in designing therapeutic strategies that include the stepwise use of medications (Fig. 2).

Treatment of Mild (Intermittent and Persistent) Asthma

Certain treatment approaches apply to all patients with asthma. Regardless of the severity of their disease, all should be educated about their disease, its potential for becoming life-threatening, and the importance of the technically correct use of medications (see below). Specific pharmacotherapy depends on the level of severity of the disease. Mild intermittent asthma can be controlled with the use of an inhaled β2-agonist as needed for spontaneous symptoms, or prophylactically before exercise or exposure to allergen (such as cat) or other noxious stimuli (such as smoke). These medicines provide rapid relief from bronchospasm by relaxing airway smooth muscle. For prophylaxis, the β2-agonist should be given 15–20 minutes before the exposure/exercise and then can be continued every four hours as needed. If patients need to use their β2-agonist on a daily basis, then they should be considered to have mild persistent asthma, and other medications must be added.

There has been some debate over whether the regular use of a β2-agonist would provide better relief for mild asthmatics than an as-needed regimen. Recently this question was addressed in a multicenter study by Drazen et al. (31). In this study, 255 patients with mild asthma and airway hyperreactivity were divided into two groups and followed for 16 weeks. One group received regular doses of albuterol via a metered dose inhaler (MDI), two puffs four times a day, while the other group received a placebo MDI on the same schedule. Both groups were also given an additional MDI containing albuterol to use on an as-needed basis. At the end of the study no significant difference between the groups was seen in PEFR variability, forced expiratory volume in one second (FEV_1), use of as-needed albuterol, symptom scores, quality-of-life scores, or airway hyperreactivity. Therefore, a regularly scheduled β2-agonist offered no advantage in mild asthma over an as-needed regimen, and given the additional costs and inconvenience associated with scheduled β2-agonist use, the as-needed use is clearly preferred.

Current inhalers use a chlorofluorocarbon (CFC)-based propellant system (30). This is problematic, in that CFCs have been implicated in depleting the ozone layer. As a result, CFCs have been banned by international treaty, although medications were granted an exception. Nevertheless, CFCs in medications will likely be phased out in the near future. As a result, new delivery systems will soon be instituted to replace traditional CFC-based inhalers. One albuterol MDI formulated with a hydrofluoroalkane 134a (HFA) propellent is already available; this propellant has no effect on the ozone layer. Other delivery systems, such as dry powder inhalers, are also anticipated. These changes will be carefully mon-

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[1] Reference 30 can be downloaded from the Internet at *"http//www.nhlbi.nih.gov/nhlbi/lung/asthma/prof.asthgdln.htm"*. This is a 1.0 MB file.

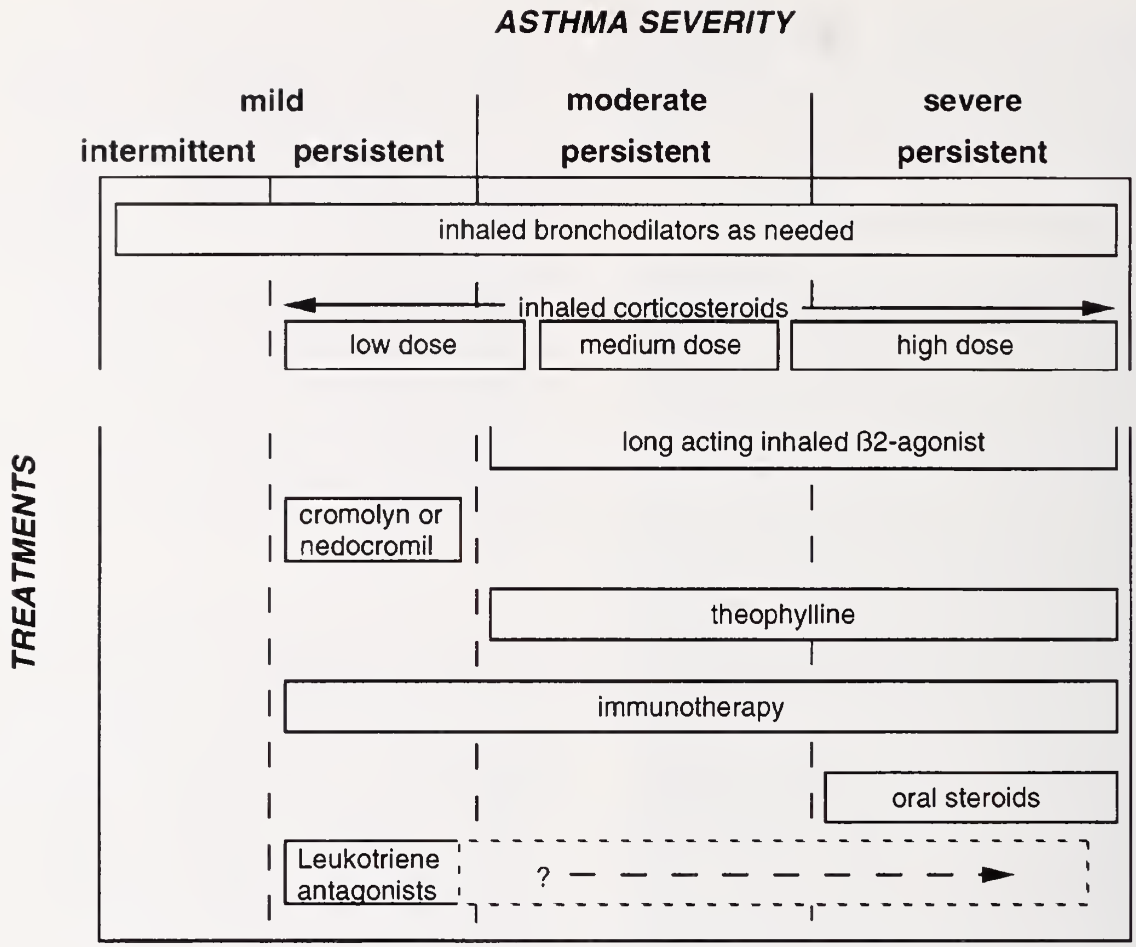
ASTHMA SEVERITY
mild
intermittent
persistent
moderate
persistent
severe
persistent
TREATMENTS
inhaled bronchodilators as needed
inhaled corticosteroids
low dose
medium dose
high dose
long acting inhaled ß2-agonist
cromolyn or nedocromil
theophylline
immunotherapy
oral steroids
Leukotriene antagonists
?

Fig. 2. Suggested use of pharmacotherapies in asthma based on asthma severity. Reproduced with permission from (2) and modified to include the most recent NHLBI guidelines (30).

itored so that new formulations will deliver similar doses compared to the original delivery systems.

Several recent studies have indicated that the earlier anti-inflammatory therapy is instituted the better the lung function over time (32–34). Given this information, in mild persistent asthma, it may become standard therapy to add anti-inflammatory medication to the bronchodilator, even in patients with mild disease. This is sometimes referred to as adding a disease controller (anti-inflammatory medication) to a disease reliever (β2-agonist). For mild persistent asthmatics, anti-inflammatory therapy can be undertaken with a low dose, either inhaled corticosteroid (see Table), or inhaled cromolyn or nedocromil. In children, these latter two nonsteroid medications may be preferred initially before instituting inhaled corticosteroid therapy. Cromolyn and nedocromil were originally thought to stabilize mast cells; however, it now appears that other as yet unknown mechanisms are involved in their efficacy.

Within the past year, a new class of medications, the leukotriene inhibitors, has become available for oral administration, and appears to be useful for the treatment of mild to moderate asthma (35, 36). Leukotrienes, generated from arachidonic acid via specific enzymatic modifications, have long been known to have potent and prolonged bronchoconstrictive properties. For example, in aspirin-sensitive asthmatics, leukotriene levels in the urine are quite elevated, and rise even further following aspirin challenge. In the airways, leukotriene D_4 (LTD_4) appears to be primarily responsible for this activity and acts on a specific receptor found on airway smooth muscle. It is therefore not surprising that the pharmaceutical industry has developed several medications to interfere with the generation or action of these leukotrienes. Two different types of leukotriene inhibitors can now be prescribed: one that blocks the synthesis of leukotrienes (by inhibiting the activity of an enzyme, 5-lipoxygenase (5-LO), required early on in the leukotriene synthesis cascade) and another that competitively blocks the LTD_4 receptor on smooth muscle and other cells. Zileutin (Zyflo, 600 mg four times daily) is the first of the 5-LO inhibitors to become available, while zafirlukast (Accolate, 20 mg bid) is the first of the LTD_4 receptor antagonist group to be approved. Others within the LTD_4 receptor antagonist group, or "lukast" family, including monelukast and pranlukast, are undergoing clinical trials. To date, these medications have been shown to prevent bronchoconstriction due to cold dry air, exercise, allergen provocation, and aspirin ingestion in aspirin-sensitive asthmatics, and to provide cost-effective reductions in the use of other medicines and health care-related costs. Now that these medications are available in the United States, the NHLBI guidelines (30) have added leukotriene inhibitors to the list of suggested treatments for mild persistent asthma. However, results of ongoing studies may eventually show these drugs to have effects in more severe forms of asthma.

Treatment of Moderate Persistent Asthma

As with mild persistent asthma, treatment is directed at the underlying inflammation (2). Therefore, in moderately severe asthmatics, anti-inflammatory therapy needs to be instituted with medium dose inhaled corticosteroids (see Table). If, after instituting therapy, the patient continues to have symptoms, then the dose of inhaled corticosteroids can be increased. Alternatively, a long acting β2-agonist (e.g., salmeterol [Serevent 2 puffs twice daily]) or a sustained release theophylline preparation may be added, either alone or with an increase in the corticosteroid dose. The long acting β2-agonists are often useful for patients in situations requiring long periods of strenuous exercise, such as athletes. Also, salmeterol can be used to help reduce the amount of inhaled or oral corticosteroids required for control of disease. A nighttime dose of theophylline or long acting β2-agonist may help prevent nocturnal symptoms. For acute exacerbations of asthma, a short course of oral corticosteroids may be prescribed, the inhaled corticosteroid dose may be increased, or salmeterol may be added. In a study by Woolcock et al. (37), 738 poorly controlled asthmatics on 1000 μg/day (24 puffs) of beclomethasone were randomized to receive either salmeterol 50 μg (1 puff) twice daily or 100 μg (2 puffs) twice daily or to have their inhaled corticosteroid dose doubled (to 2000 μg/day [48 puffs]) for 24 weeks. At the conclusion of the study there was no difference in exacerbation rates or methacholine reactivity among the three groups. However, there were greater improvements in PEFR, FEV_1, percentage of symptom-free days and nights, and percentage of days and nights that required no rescue medication in the two salmeterol groups than the beclomethasone alone group (no difference was noted between the two salmeterol groups). Therefore, the addition of salmeterol may provide more improved pulmonary function and symptom control than increasing the inhaled corticosteroid dose. Moderately severe asthmatics should still be provided with a short-acting inhaled β2-agonist to use on an as-needed basis (but not more frequently than every 4–6 hours). The requirement for a short-acting inhaled β2-agonist more than three times a day, or use of an entire β2-agonist canister in less than a month, indicates inadequate asthma control, and usually necessitates an increase in the anti-inflammatory medication dosage.

Treatment of Severe Persistent Asthma

In the severe asthmatic, therapy should be undertaken as for the moderate patient; however, a higher dose of inhaled corticosteroids should be employed (see Table). Corticosteroids have multiple mechanisms of action. With respect to the mechanisms of eosinophil recruitment mentioned above, they have been shown to suppress eosinophil recruitment by inhibiting cytokine and chemokine production, as well as by causing eosinophils to undergo apoptosis (21). Airway edema and bronchorrhea are also abrogated with the use of these medications. As a result, corticosteroids remain the mainstay of asthma therapy. While head-to-head studies comparing the relative efficacy of various steroid preparations for the treatment of asthma are sorely lacking, *in vitro* and *in vivo* studies suggest that fluticasone and budesonide are the most potent. A sustained-release preparation of theophylline or a long-acting β2-agonist may again be used, and may be helpful in reducing the total daily steroid dose. Oral corticosteroids are often required in these patients and can be given in short bursts without tapering, or if longer courses are required, tapered over a period of weeks. Traditionally, oral steroids have been given as a daily dose with breakfast to reduce adrenal suppression. One study, however, found that giving oral steroids at

TABLE

*Daily Doses of Inhaled Corticosteroids Used to Treat Adults with Asthma**

	Low dose µg/day	Medium dose µg/day	High dose µg/day
Beclomethasone Dipropionate Vanceril, Beclovent (42 µg/puff) Vanceril DS (84 µg/puff)	168–504	504–840	> 840
Flunisolide Aero-BID, Aero-BID-M (250 µg/puff)	500–1000	1000–2000	> 2000
Fluticasone Propionate Flovent-44 (44 µg/puff) Flovent-110 (110 µg/puff)	88–264	264–660	> 660
Triamcinolone Acetonide Azmacort (100 µg/puff)	400–1000	1000–2000	> 2000
Budesonide** Pulmicort turbuhaler (200 µg/inhalation)	200–400	400–600	> 600

*These agents are typically used every 12 hours

**Not yet available in the United States

Modified from the NHLBI guidelines of 1997 (see text for the web site address)

3 PM led to greater efficacy in asthma control, with no apparent increase in adrenal suppression (38). Sometimes patients are placed on a corticosteroid taper, and then reach a dose below which they experience symptoms. These patients should have their steroid dose decreased as much as possible, and then have the dose converted to an alternate day schedule, as this may reduce corticosteroid side effects. For those patients requiring chronic steroid therapy in whom osteoporosis is of concern, it may also be beneficial to provide supplemental calcium (1500 mg/day). Moreover, in postmenopausal women, the use of estrogen and diphosphonates such as fosimax (39) should be considered. All patients with severe asthma should be referred to an asthma specialist for control and monitoring of their disease.

Asthma Education Strategies

A critical component of asthma management is an educated patient (31). Therefore, it is of the utmost importance that every asthmatic be taught about the disease and become an active participant in their own care. In this approach, the patient takes a more active role, and is asked to monitor symptoms and respond with therapeutic decisions, much like diabetics who alter their insulin dose depending on blood glucose monitoring. Central to this approach is allowing the patient sufficient time to ask questions. Most patients will be concerned about their medications (especially if an inhaled or oral corticosteroid has been prescribed). They often will ask about safety issues, whether the medication is habit-forming, and if they will develop tolerance to the medication. In discussing the diagnosis of asthma, it is important to stress the chronic nature of the disease. The physician must describe the various symptoms the patient may experience. The triggers of asthma (both those that are specific to the patient as well as more general ones) should be explained, along with avoidance strategies.

One of the most important aspects of the education program is teaching patients when and how to properly use their medications. While most physicians think their patients understand the correct use of inhalers, this may not actually be the case. In a study of patients admitted to two major inner city teaching hospitals for asthma exacerbations, it was found that most patients had used their β2-agonist (either via an MDI or a nebulizer) within 24 hours of admission (40). Frighteningly, on average, these patients had used 44.8±7.8 puffs of their β2-agonist MDI in the preceding 24 hours. But only 10–15% of the patients had used the medication either one or two hours prior to admission. This is a concern, because the only medication that can provide acute relief of bronchospasm is the β2-agonist, yet it appears that most patients fail to use this medication when their asthma is most severe. Even more disconcerting is the fact that fewer than 30% of patients had any kind of "action plan" outlined by their care giver so that they would know what to do as their asthma worsened. Obviously, when it comes to education in asthma treatment, there is much room for improvement.

Proper Use of Inhalers, Spacers, and Home PEFR Monitoring

Using an MDI correctly can be difficult for patients, but is very important for appropriate delivery of medication to the lungs. In the study of inner-city asthmatic patients (40), it was found that 80–90% of the subjects knew to actuate the MDI only once at a time and to inhale with each actuation. However, just over half of the subjects took a slow inhalation or held their breath after inhaling. Worse yet, less than a quarter of the patients performed the open mouth technique correctly (placing the MDI 2-3 fingerbreadths from the open mouth, actuating the MDI and then inhaling slowly, holding their breath for 10 seconds). Only a scant 15% of subjects were able to perform all the components of MDI usage correctly. Therefore, while the correct medication may be prescribed, it is often used incorrectly.

To improve delivery of nebulized medications from an MDI into the lower airways, especially for corticosteroids, it is helpful to use a spacer. Because these devices remove the larger particles which otherwise tend to become trapped in the oropharynx, they reduce the incidence of *Candida* infections (oral thrush) as well as dysphonia. Spacers also improve the distribution of aerosol from the MDI, and compared to using the MDI without a spacer, can nearly double the amount of medication that reaches the lung. This has translated, in one study (41), to improving pulmonary function by almost 10%. Additionally, the use of a spacer lessens the need for fully coordinated patient effort with actuation of the MDI. Therefore, a spacer device should be used whenever a medium or high dose of inhaled corticosteroid is prescribed. Severe asthmatics may need a home nebulizer for delivery of short-acting β2-agonists, especially during exacerbations, but this is becoming less common. Although for a well-trained patient, nebulizers are not necessarily better at delivering medication than an MDI, in a severe attack, patients often lack the coordination required for use of an MDI. In this situation, a nebulizer can be beneficial.

Because patients and physicians are often inaccurate at estimating the severity of an exacerbation of asthma, it is important that an objective measure of airway obstruction be used. In the office or hospital setting, useful information on the severity of obstruction can be obtained with pulmonary function testing and the determination of PEFR, and/or the ratio of FEV_1 and forced vital capacity (FVC). Reversibility of obstruction can also be readily assessed by repeating these measurements after administration of a β-agonist. However, for measurements of lung function to be useful on a daily basis, the patient must be able to perform these tests at home. For this, PEFR can be measured easily at home with the use of a peak flow meter, a device that can be purchased for $30 or less. All asthmatics with moderate or severe disease should have a peak flow meter, and should be instructed in how to monitor their PEFR (30). This includes keeping a diary of values and using the meter daily in the morning and evening, before inhaling their bronchodilator. Patients should know how to adjust their anti-inflammatory medication whenever their PEFR drops below 80% of their baseline and to call their doctor when their PEFR is below 60% of predicted. With this type of early warning system and appropriate medical intervention, most hospitalizations and emergency room visits can be avoided.

The Role of Genes, IgE, and Immunotherapy in Asthma

Asthma Genetics

Asthma is a genetically complex disease, in that there is no single gene responsible for the disease. Interestingly, total IgE and asthma phenotype have been linked (42–44), and research into the likely genes has concentrated on this relationship. Currently, there are at least 15 regions in the human genome that have been linked to asthma and atopy (44, 45). These potential "asthma genes" are located on five chromosomes. These chromosomes also encode the genes for various components of the immune system that are involved with atopy and IgE production. For example, one candidate gene is located in the region on chromosome five where IL-4 is encoded. Another is located on chromosome 12 near the gene for IFNγ, a cytokine that antagonizes the effects of IL-4. Near the gene for the high-affinity receptor for IgE on chromosome 11 is another candidate gene. A fourth locus can be found on chromosome 6, in the region of the class II MHC (HLA-D), a molecule involved in antigen presentation. One additional region has been found on chromosome 14, near the gene for the T cell receptor. While more work still needs to be done, it is clear that asthma arises from multiple genetic factors as well as environmental exposure.

The Role of IgE

Since allergies exacerbate atopic asthma, inhibiting or perhaps even eliminating allergic reactions should reduce the number of exacerbations due to allergen exposure and possibly lead to a cure for asthma. It has been known for about 30 years that IgE is the immunoglobulin responsible for allergic reactions. This immunoglobulin binds to high-affinity IgE receptors ($Fc_{\varepsilon}RI$) on mast cells and basophils. Antigen cross-linking of receptor-bound IgE on these cells leads to their degranulation, with subsequent release of histamine and other mediators associated with the immediate phase of the allergic response. Therefore, therapy to eliminate IgE antibody, or to block IgE binding to its receptor, would have the potential of preventing allergic responses. Regarding the former approach, it is now known that the cytokine IL-4 is a critical signal for B cells to switch to IgE production. Efforts to antagonize IL-4 have already entered clinical trials (46), and may ultimately be capable of suppressing IgE synthesis *in vivo*. Regarding the latter approach, humanized anti-IgE antibodies have been made that bind to the domains on IgE that are necessary for its binding to $Fc_{\varepsilon}RI$, and therefore block binding of IgE to its receptor. Importantly, these antibodies are unable to bind to IgE that is already attached to the high affinity receptor, because this site is presumably hidden once IgE is bound. If this were not the case, the anti-IgE antibodies could cross-link surface bound IgE and lead to widespread basophil and mast cell degranulation and anaphylaxis. Two such anti-IgE antibody preparations are undergoing clinical trials, and appear to be safe and extremely effective in lowering serum free IgE concentrations by as much as 100-fold (47, 48). An added benefit of this therapy is that the level of $Fc_{\varepsilon}RI$ on basophils is also dramatically reduced, making it more likely that IgE-dependent mediator release responses will be altered *in vivo*. Additional studies, already underway, should soon determine whether these treatments will lead to clinical improvement of asthma.

Allergen Immunotherapy

The role of allergen immunotherapy in asthma remains controversial (49). Most well-controlled trials show some degree of efficacy. Now that allergens are becoming standardized, the number of studies showing efficacy in the treatment of asthma is increasing: A recent, multi-center double-blind placebo controlled study in adults by Creticos et al. (50) demonstrated that ragweed immunotherapy decreased asthmatic symptoms during the first of two consecutive ragweed seasons. While the results were significant, the extent of relief was modest. In a study of asthmatic children, Adkinson et al. (51) found no significant difference between the immunotherapy treated and placebo groups. Unlike the Creticos study, however, the Adkinson study employed an even more "real world" approach in that the immunotherapy administered was designed to cover most, if not all, of the relevant allergens for each of the children. Interestingly, both studies found that all groups improved substantially during the study. This appears to be due in part to the close follow up (e.g., every two weeks in the Adkinson study) that was afforded to all study subjects.

In our view, classical immunotherapy should be considered in patients who have failed to respond to environmental (e.g., dust mite precautions) and pharmacologic therapies (2). Patients with symptomatology related to allergen exposure may benefit most from a trial of immunotherapy. This is true, however, only in those situations in which the allergens are well defined, as in the case of house dust mites or grass, ragweed or tree pollens. In certain situations, as in sensitivity to molds, the allergens are not defined and immunotherapy cannot be recommended. In other situations, such as in the common sensitivity to animal danders in the home or to laboratory animals in science workers, the allergens are becoming defined, and their efficacy is currently being tested.

A newer form of immunotherapy, called peptide immunotherapy, has recently been undergoing clinical trials (52). This treatment involves using a specific piece of the allergenic protein, rather than the whole protein, that maintains its ability to interact with macrophages and T cells. This may permit a much shorter duration of therapy. Also, because this fragment is too small to cross-link IgE, it should allow for a safer form of immunotherapy. So far, clinical trials with cat and ragweed-based peptides have shown modest efficacy; this has yet to be compared head-to-head with traditional immunotherapy.

Summary

Asthma represents a multifaceted disease that involves activation of a variety of cells including mast cells and T cells. This activation leads to infiltration of eosinophils into the lungs and chronic inflammation. Therapy is thus directed at preventing or reversing this inflammation as well as the accompanying bronchospasm. Novel therapeutic approaches directed against cytokines, chemokines, adhesion molecules, IgE, and other targets are being designed to help prevent allergic triggers from initiating the cascade of events leading to an asthma flare. By combining allergen-directed and pharmacologic strategies with patient education, physicians can help their asthmatic patients live normally active lives.

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Current Events and Bioethical Concerns in Physician-Assisted Death

Jeffrey T. Berger, M.D., F.A.C.P.,[1] Fred Rosner, M.D., F.A.C.P.,[2] and Allen J. Bennett, M.D.[3]

Abstract

In June 1997, the Supreme Court of the United States found that the Constitution does not guarantee a right to physician-assisted suicide, thereby allowing states the opportunity to variously prohibit or permit such practice. The Court's findings notwithstanding, physician-assisted death remains a topic of intense medical, legal and philosophical discussion. Principled discourse variously supports both an ethical prohibition against assisted death and an ethical obligation to help some patients achieve death. Both theoretical and practical concerns are raised by the practice of physician-assisted death. This essay reviews recent events and developments concerning assisted suicide and euthanasia. The discussion which follows was generated by the members of the Committee on Bioethical Issues of the Medical Society of the State of New York and builds upon a previous Committee report. **Key Words:** Assisted suicide, euthanasia, bioethics, patient rights.

Introduction

In this essay, the term "physician-assisted death" is used to describe both assisted suicide and active euthanasia in circumstances where little relevant distinction exists. Principled discourse variously supports both an ethical prohibition against assisted death and an ethical obligation to help some patients achieve death. Because of a 1997 Supreme Court decision that will be discussed later in this essay, it is likely that some states, under certain circumstances, will permit physician-assisted suicide. This raises both ethical and pragmatic issues that must be addressed.

The literature in support of assisted death often argues that physicians must respect the choices of terminally ill patients, relieve their prolonged and unnecessary suffering, and give reassurance to patients that they control their own dying. Opposing arguments find assisted death incompatible with the moral and professional obligations of physicians to protect and preserve life, and raise concerns about patient mistrust of physicians and the potential weakening of society's commitment to provide optimal terminal care. Slippery slope concerns of voluntary euthanasia leading to nonvoluntary euthanasia are countered by those who propose criteria to regulate assisted death (1). Still others persuasively argue that such regulation is impossible (2).

These points have been offered and challenged in many publications (3). This essay reviews recent developments concerning physician-assisted suicide and euthanasia, and discusses the implications of impending practice. The discussion which follows was generated at a meeting of the Committee on Bioethical Issues of

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[1] Assistant Professor of Medicine, SUNY Stony Brook School of Medicine, Director of Clinical Ethics, Winthrop-University Hospital, Stony Brook, NY, [2] Director, Department of Medicine, Mount Sinai Services at Queens Hospital Center, Jamaica, NY, Professor of Medicine and Assistant Dean, Mount Sinai School of Medicine, New York, NY, and [3] Clinical Instructor of Medicine, Mount Sinai School of Medicine, New York, NY. Address correspondence to Jeffrey T. Berger, M.D., Winthrop-University Hospital, 222 Station Plaza North, Suite 518, Mineola, NY 11501.

the Medical Society of the State of New York and builds upon a previous Committee report (4).

Professional and Popular Opinion

Many recent studies examine physician and public attitudes toward assisted death. In Washington State, 48% of physicians surveyed believe that euthanasia is never justified, while 42% disagreed (5). In Oregon, 60% of physicians surveyed believe that assisted suicide should be legal in some cases, and 46% of these physicians said they might participate in assisted suicide if it were to become sanctioned legally. The survey also found that 7% of responding physicians had already assisted in at least one suicide (6). A recent study suggests that most New Jersey physicians would not participate in assisted suicide (7). A survey of 250 oncologists revealed that 18% had participated in assisted suicide and 4% in active euthanasia (8). About 20% of these physicians favored legalizing assisted death. Fifteen percent of Canadian physicians surveyed reported that they had been asked to assist in at least one suicide and 20% had been asked to provide active euthanasia (9). Sixteen percent of Washington State physicians surveyed received at least one patient request for assisted death (10). Patients' concerns that are perceived most often by physicians in these requests tend to be nonphysical, such as loss of dignity and control (10). In a sampling of 2,000 Canadian citizens, about 60% support physician-assisted death for competent persons with poor medical prognoses (11). However, studies have not described the durability of such support or the stability over time for requests for assisted death (12).

Global Experiences

The Netherlands is the major source of actual experience with the practice of physician-assisted death. Although euthanasia remains technically illegal in the Netherlands, it has been condoned by the courts since 1991 with certain procedural requirements. These stipulations include a persistent and voluntary patient request for death, unbearable suffering, and agreement among the primary and corroborating physicians. Assisted suicide for psychological suffering is not prohibited (13). The two most common reasons for a patient to request euthanasia are loss of dignity and pain (14).

Recent data suggest that only 23% of Dutch physicians have participated in assisted death and that most requests for death are denied (15, 16). Although several hundred people are euthanized each year without a clear voluntary request (17), the rate of nonvoluntary euthanasia seems to have decreased slightly over the past 5 years (16). These authors (16) noted that assisted suicide remained constant and low. The rate of voluntary euthanasia increased from 1.7 to 2.4% of deaths. Nonetheless, it is reported that some concerned Dutch citizens are now carrying "passports for life" to protect themselves from nonvoluntary euthanasia in the event of a medical emergency (18).

Physician compliance with reporting requirements has been poor, although improving (19). A study examining assisted death practices between 1990 and 1995 found that the frequency of instances of assisted death reported to the authorities increased from 18% to 41%, although a majority of cases still remain unreported (20). Reasons given for notification of noncompliance include the time-consuming and burdensome reporting requirements, a desire to protect family members from judicial inquiry, and concerns that reporting violates patient privacy (20).

In 1995, a Dutch court dismissed a murder charge against a physician who admitted that he had killed a genetically defective 3-week-old infant (21). The court acknowledged that the physician did not fulfill the euthanasia requirements, but supported the action by ruling that the physician had no other choice.

Australia's Western Territories became the first jurisdiction in the world to explicitly legalize voluntary active euthanasia (22). The Rights of the Terminally Ill Act came into force on July 1, 1996, but implementation was delayed by Territorial Supreme Court and Federal High Court reviews. The Act allowed terminally ill patients who suffer from intractable pain or distress to request of their physician either assisted suicide or euthanasia. At least nine days had to elapse between the first and second requests. The physician had to certify that medical interventions cannot provide relief of suffering to the patient's satisfaction. A second physician had to concur and a psychiatrist had to see the patient to exclude treatable depression. The first case of legally permitted euthanasia occurred on September 26, 1996 (23). In March 1997, a Federal Parliamentary bill overturned the Territories' Act, thereby reinstating legal prohibitions against assisted suicide and euthanasia.

National Events

In March 1996, the United States Ninth Circuit Court of Appeals found unconstitutional a

Washington State statute prohibiting assisted suicide (24). The Court asserted that a liberty interest exists in directing or hastening one's death. The Court found no significant ethical or legal difference between withdrawing life-sustaining interventions and assistance in suicide. The Court viewed as equivalent death due to the administration of an analgesic in doses intended to relieve pain and death following use of an analgesic in doses intended to cause death.

One month later, the United States Second Circuit Court of Appeals overturned a New York State law prohibiting assisted suicide (25). The Court found the New York law unconstitutional on grounds that it violated the Constitution's equal protection clause. In an opinion with which we disagree, Judge Miner found "similarly circumstanced" patients whose lives are dependent on life-sustaining devices and terminally ill patients who wish to be dead. The Court did not clarify the important differences between patients who would already be dead if not for modern medical interventions, and those who would be dead only with medical interventions to hasten death. Judge Miner wrote ". . . by refusing to accept [life-sustaining interventions] in the first place, a patient hastens his death by means that are not natural in any sense." The Court asked ". . . what interest can the state possibly have in requiring the prolongation of a life that is all but ended?" The Court was unclear in defining when life is actively prolonged.

These two Circuit Court decisions were appealed to the United States Supreme Court. In June 1997 a unanimous Court decision, which included five separate concurring statements, upheld the states' right to prohibit physician-assisted suicide (26). The Court found that suicide or assistance in suicide is not a fundamental liberty interest, in that suicide runs contrary to centuries of common law and our nation's history and tradition disapproving of suicide. Chief Justice Rehnquist, in delivering the opinion, maintained the important distinction between the refusal of medical intervention and medically assisted suicide. Administration of unwanted treatment has long been considered battery. The Due Process Clause of the Constitution protects this right of refusal. The opinion states that "the decision to commit suicide with the assistance of another may be just as personal and profound as the decision to refuse unwanted medical treatment, but it has never enjoyed similar legal protection. Indeed the two acts are widely and reasonably regarded as distinct." The discontinuation, by a physician, of life-sustaining interventions is considered passive euthanasia. The Court did not address active euthanasia, which involves an action that kills a patient sooner than the disease process would dictate.

The Court enumerated the legitimate state interests upon which suicide prohibitions may be based. These "include prohibiting intentional killing and preserving human life; preventing the serious public health problem of suicide, especially among the young, the elderly, and those suffering from untreated pain or from depression or other mental disorders; protecting the medical profession's integrity and ethics and maintaining physicians' roles as their patients' healers; protecting the poor, the elderly, the disabled, the terminally ill, and persons in other vulnerable groups from indifference, prejudice, and psychological and financial pressure to end their lives; and avoiding a possible slide toward voluntary and perhaps even involuntary euthanasia."

Justice Rehnquist encouraged continuation of this debate, as did Justice Souter, who in a separate concurring opinion suggested that state legislatures offer a preferred venue for deliberation on, and regulation of, assisted suicide. Justice Souter also accepted that, for competent and terminal patients, physician prescription of medications with intent to effect death is within the standards of medical practice.

Justice Stevens, in a separate concurring opinion, also called for " . . . vigorous debate about the outcome of particular cases . . ." and suggested that " . . . upholding a general statutory prohibition of assisted suicide does not mean that every possible application of the statute would be valid." He continues ". . . some individuals who no longer have the option of deciding whether to live or to die because they are already on the threshold of death have a constitutionally protected interest that may outweigh the State's interest in preserving life at all costs." Justice Stevens suggested that for patients whose vulnerabilities are not exploited and who are not depressed, individual interests in accomplishing suicide may, in fact, outweigh states' interests in prohibiting it.

In November 1997, following the Court's decision, Oregon voters reaffirmed a 1994 referendum permitting physician-assisted suicide (27) by a two-thirds majority compared to the earlier vote in favor of 51%. The referendum has been implemented, and the state of Oregon is collecting data on the use of assisted suicide.

These developments, when considered en bloc, suggest a shift in general and legal sentiment toward permitting physician-assisted death. The implications of this evolution are enormous. Several of these implications are now discussed.

Concerns and Implications

Is there a principled distinction between assisted suicide and active euthanasia?

Some proponents of legalized assisted suicide argue that prohibiting active euthanasia protects patients, since in assisted suicide, the patient, not the doctor, controls the final and defining action, for example, the ingestion of a lethal dose of medication (28). This argument supporting assisted suicide is predicated on a specious assumption about physician support of patient autonomy. The assumption is that physicians will be willing to assist in implementing the decisions of patients capable of self-administration of medication (assisted suicide), while rejecting the same ethical imperative in cases where patients who have reached the same decision are incapable of self-administration of medication (active euthanasia).

In fact, neither the principle of autonomy nor the beneficence-derived obligation to relieve suffering serves to clarify the line between assisted suicide and active euthanasia. Proposals for regulating assisted death incorporate these elements through requirements of intractable pain and repeated voluntary patient requests for death. Consider a patient making a reliable (i.e., without treatable depression) and voluntary request for assisted suicide. Pills are given to the patient, who places them in his mouth and swallows them. Consider a similar patient who is unable to swallow due to oral cancer. He has a gastrostomy tube. Pills are given to the patient, who crushes them and then flushes them down the tube. Consider another patient who is too weak to crush the pills. Someone assists in crushing the pills and places them in a syringe for the patient to administer through the tube. Another patient may be too weak to push the full amount of medication through the tube, and after he begins to do so, someone else assists in completing the injection of the medication bolus. A patient may be too weak to participate physically in any way in the ending of his life, even though he clearly wishes to do so, so someone else prepares and administers the medication through the tube. In all these scenarios, patient self-determination is respected and relief of suffering is achieved. The same principles underlie both assisted suicide and euthanasia, and offer no clear-cut points distinguishing the two activities. Is the ingestion of a lethal substance by a participating but unwitting, demented patient considered to be suicide, euthanasia or something else? If the family supports such activity, is it surrogate-mediated suicide or nonvoluntary euthanasia? How and where will the line be drawn between permissible and impermissible actions? Could such restrictions be enforced, particularly if doctor-patient confidence and patient privacy are respected? These questions have not been considered adequately to date and require further study.

Would society's vulnerable members be pressured to accept assisted death if it were legal? Would society be morally obligated to provide greater health and social supports to protect these people from such pressure?

The Supreme Court recognized the risks the practice of assisted death would present for vulnerable patients. If dying becomes a choice, then living is no longer a simple fact but also a choice which may be subject to scrutiny and justification. Most seriously ill patients desire both a limit on the duration of dependency on their families and a limit on their personal expenditures on medical care if they become debilitated. How treatment preferences might change with legalized assisted death is unknown and subject to debate (29). Even if legal requirements for voluntary patient requests for death are in place, voluntariness is relative and difficult to verify. Elderly patients might choose an early death to avoid burdening relatives, or in response to subtle or overt family pressure. Patients' motivations for many health-care decisions are left unexplored by health professionals. However, expanding patient choice to include assisted death also amplifies the potential for abuse.

The lack of a comprehensive approach to health care has been a significant impetus for the popular support of physician-assisted death in this country. Inadequacies in palliative and hospice care and the lack of integrated mental health services must be remedied before a coherent argument can be developed which might alter social policy to permit assisted death. Even so, such a radical change would pose significant risk to many patients while likely benefitting a very small number. Prudence requires implementing improvements that would benefit many, if not most patients before instituting a policy of physician-assisted death.

Health care in the Netherlands, considered by some to be more ideal than our own, is universal, and vertically integrated, and features a high degree of physician-patient continuity. It has been reported, however, that some physicians in the Netherlands have broadened the scope of their professional activity to include nonvoluntary euthanasia. This breach has judicial support despite its clear violation of legal requirements (17, 19, 21). How the practice of assisted death would evolve within the seriously flawed American health system is anyone's guess. American physicians in small towns or in rural communities are less likely to participate in assisted suicide than physicians in larger communities (6), suggesting perhaps an inverse relationship between continuity of care and demand for assisted death. Proponents of physician-assisted death recognize continuity of care as important to successful policy. Unfortunately, rapid changes in health care delivery in this country render more elusive a long-term doctor-patient relationship.

How would permitting assisted death affect the socioeconomically disadvantaged members of our society?

Inequitable distribution of health care and latent race-related practices have engendered a high level of suspicion of the health system by some ethnic minorities and the economically disadvantaged. How might the practice of assisted death cross-react with these deficiencies? This concern is central to practical deliberations, because if assisted death is permitted, abstract discussions will give way to considerations of actual patients, many of whom are not well empowered.

Perhaps the racial minority best studied in terms of health care is the African American. Six sources of African American distrust of the medical profession are: the use of slaves as medical research subjects, the Tuskegee syphilis experiment, abuses from sickle cell screening and anti-minority sterilization initiatives in the 1970s, suspicion that the AIDS epidemic was manufactured with genocidal intent, and government-sponsored research suggesting a genetic link to violent behavior (30). Birth control initiatives over recent decades are viewed as genocidal by many African Americans (31). Minority Americans have a poorer quality of health than do majority whites, and African Americans receive less intensive health care even when access to care and preferences for treatment are considered (32). It is not surprising that African Americans place a greater emphasis on security, survival and community than white people, who place greater value on personal autonomy (31). It follows that African Americans are less likely to decline life-sustaining treatment regardless of illness severity (33), and African Americans and Hispanic Americans are less likely than non-Hispanic whites to desire assisted death (33).

This sociomedical history must be considered in assisted death discussions. For example, the Dutch experience demonstrates that requirements for informed consent for euthanasia are not rigidly upheld. Since the quality of informed consent and patient knowledge of the right to refuse treatment correlates with level of education (34), the potential exists for socioeconomically disadvantaged patients to suffer disproportionately from abuses in the regulation of assisted death. Furthermore, minority patients may be at greater risk for having to defend a decision to decline assisted death, because minority patients have less control over their confidential information in health care institutions (35).

Deeply rooted minority mistrust of the health care system would be compounded if the practice of assisted death were codified, such that it was followed by minority avoidance of health care. Patient suspicion of possible undertreatment by physicians, particularly within managed care environments, could be magnified if physician-assisted suicide were permitted. The relatively small number of patients likely to receive physician-assisted death measured against the large numbers of potentially affected minority patients suggests net harm for the aggregate. Furthermore, physicians' motives in advocating nontreatment or assisted death would be suspect.

How would pain management in terminal care be affected by the legalization of assisted death?

Deficiencies in the provision of analgesia and palliative care are well recognized. Will bureaucratization of assisted death improve or worsen terminal pain management? In Oregon, following the introduction of a bill to permit assisted suicide, demand for hospice services increased (36). Although the Supreme Court clearly accepted aggressive analgesia when medically appropriate, regulatory requirements may cause physicians to palliate patients' pain less vigorously. Physicians may underdose analgesic medications out of concern that a terminal patient's accidental demise might be mistakenly attributed to a case of intended and illicit euthanasia. Could cases where physicians intentionally euthanized patients outside of protocol be differentiated reliably from cases where patients expired from unintended narcotic effects? This ambiguity is evidenced in recent Dutch data where unclear or inconsistent physician behavior was implicated in 2% of all deaths (16). Current efforts to improve physicians' treatment of pain should continue. However, physicians' desire to improve pain control might be overshadowed by their concern about being accused of committing unsanctioned euthanasia. Nurses may feel similarly at risk.

How would current mechanisms for health decision making serve incapacitated patients in assisted death?

Advance directives—commonly living wills and health care proxies—are promoted as important tools for preparing for end-of-life decisions. Even though the Supreme Court drew a clear distinction between the right of treatment refusal and the lack of general entitlement to assisted suicide, advance directives likely will be used by some patients to direct assisted death. However, serious problems with advance directives exist. The public has rebuffed widespread professional and governmental efforts to increase the use of advance directives (37, 38). But even while available, these tools do not always facilitate consistency between a patient's preferences and that patient's treatment, as a result of their inaccessibility when needed and physician inattention to them (39). Moreover, physicians rarely initiate discussions about the availability of advance directives or treatment preferences, despite patient receptivity and expectation (40–44). In fact, in one study, approximately 90% of outpatients desired advance directives (45). Finally, advance directives' use for assisted death is problematic. Would patients use them as protection from euthanasia? If so, current access difficulties could have more serious sequelae. Consequences of imprecise interpretation of living wills would be magnified. Would a health proxy agent be disqualified from care decisions for not following a patient's prior wishes for euthanasia? The complex interrelationship of assisted death and current decision-making methods must be considered.

The Committee on Bioethical Issues of the Medical Society of the State of New York

The members of the Committee on Bioethical Issues of the Medical Society of the State of New York are deeply and fundamentally divided on the moral nature and ethical appropriateness of assisted death (46). A large minority of members hold that physicians' obligation to relieve suffering is sufficiently weighty to permit assisted death as a means to such relief. These members consider it ethical for autonomous patients to actively direct and achieve their own ends.

The Committee's collective ambivalence mirrors that of physician groups elsewhere. A slim majority of members find assisted death morally offensive, while many others hold that assisting some patients in achieving death is ethically acceptable. Both groups recognize that the practice of assisted death is fraught with serious concerns. Committee members in favor of the practice of assisted death find these issues either resolvable or not prohibitive. Some members remain unconvinced that resolution of the many significant concerns can be achieved. Yet others opposed to assisted death invoke the fundamental principle of nonmaleficence. Some members believe that, while suicide is a valid personal decision, medicalization of suicide is unacceptable and damaging to the health profession. Still others believe that institutionalization of the practice of assisted death must be rejected to preserve the integrity of the medical profession and health delivery system, but that acts of physician-assisted death may not be ethically objectionable in exceptional cases.

To date, organized medicine has uniformly frowned on physician-assisted death. However, the practice of assisted suicide has already begun in Oregon, and is likely to follow in other jurisdictions. The practice of physician-assisted suicide will inevitably affect the character of the medical profession. Regardless of legal permissibility, professional organizations should continue to reject the institutionalization of the practice of assisted death, while recognizing that some principled physicians will assist in patient suicide. Dialogue within the profession should continue.

We call on governmental, professional and private agencies to work resolutely to obviate the need for patients to pursue assisted death. This effort to effect a dramatic change in the care of the dying should include:

(1) Expanding home-based hospice and institutional hospice resources, and visiting nurse services.

(2) Improving social supports and family services for persons who are elderly, seriously ill, or dying. These supports should include home health aides, companion programs, adult day programs, and respite opportunities, so that patients who wish to remain at home may do so without isolation and unchecked family stress.

(3) Expanding mental health services and coverage for improved diagnosis and treatment of depression. This would include efforts to improve primary care practitioners' skills in diagnosing and treating depression.

(4) Integrating the above resources into an efficient, responsive, and seamless process which attends to issues of ethnic and religious diversity.

(5) Increasing the emphasis on holistic care, pain management, and culturally sensitive care in health education. This education should include the training of physicians to discuss end-of-life choices and to use life-sustaining medical technologies with greater sensitivity to the wishes of patients and their families.

(6) Reevaluating current mechanisms and processes for surrogate decision making, which have proven to be inadequate and have caused patients to feel disempowered and vulnerable.

Assisted death is a moral and ethical challenge which has the potential to polarize physicians, patients, and medical and legal groups. Legal determinations notwithstanding, the ethical questions remain unresolved. Assisted death will herald an intense reexamination of the moral and philosophical relationships between killing and allowing to die, deficiencies in the health care delivery system, and the role of health professionals in society. Such reexamination—while it may cause discomfort to many—may well prove beneficial.

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Appendix

Committee on Bioethical Issues of the Medical Society of the State of New York, members: Fred Rosner, M.D., Chairman; Allen J. Bennett, M.D., Vice Chairman; Jeffrey T. Berger, M.D.; Anthony Buscaglia, M.D.; Eric J. Cassell, M.D.; Peter Farnsworth, M.D.; Abraham L. Halpern, M.D.; Michael Hutzler; Allan Jacobs, M.D.; Sanjay Jain, M.D.; Matthew Karen, M.D.; Pieter Kark, M.D.; George Kofinas, M.D.; Allison B. Landolt, M.D.; Lawrence Loeb, M.D.; Robert E. Lowenstein, M.D., Ph.D.; Patricia J. Numann, M.D.; Fernando V. Ona, M.D.; Samuel Packer, M.D.; Joel Potash, M.D.; Herman M. Risemberg, M.D.; Peter Rogatz, M.D.; Joshua Samuels, M.D.; Philip H. Sechzer, M.D., Marie Thearle.

Anesthesia for Nonobstetric Surgery During Pregnancy

Yaakov Beilin, M.D.

Abstract

Approximately 50,000 pregnant women undergo nonobstetric surgery each year in the United States. Administering anesthesia during such surgery is one of the only situations in which anesthesia impacts on more than one individual (mother and fetus) at the same time. Providing a safe anesthetic to the pregnant woman requires an understanding of the physiologic changes of pregnancy and the impact of anesthesia and surgery on the developing fetus. The following review will consider the risks to the mother and to the fetus during nonobstetric surgery. **Key Words:** Anesthesia, morbidity, obstetrics, outcome, pregnancy, surgery.

Introduction

A previous review of this topic by the author was published several years ago (1). This update is timely, since the incidence of surgery for nonobstetric procedures during pregnancy is from 0.3 – 2% (2, 3). In the United States, approximately 50,000 pregnant women undergo surgery for a nonobstetric condition each year. Even this may be an underestimate, due to surgery performed prior to clinical recognition of the pregnancy. Appendectomy is the most frequent nonobstetric operation during pregnancy. However, almost every type of surgery has been done successfully during pregnancy, including open heart procedures with cardiopulmonary bypass (4), neurosurgical procedures requiring hypotensive techniques and hypothermia (5), and liver transplantation (6).

The purpose of this article is to review the anesthetic considerations for the pregnant woman who presents for nonobstetric surgery, as they relate to both the mother and the fetus. Maternal considerations result from the physiologic changes of pregnancy that effect almost every organ system (Table 1). In order to provide safe anesthesia to the pregnant patient, one must consider not only these physiologic changes, but also when they occur during gestation and how they impact the administration of anesthesia. Fetal concerns include the possible teratogenic effects of anesthetic agents, avoidance of intrauterine fetal asphyxia and prevention of premature labor. This review will conclude with practical and clinically relevant recommendations.

Physiologic Changes of Pregnancy

Respiratory System

Due to increased progesterone levels during the first trimester, minute ventilation is increased by almost 50% and remains at this level for the remainder of the pregnancy. Because anatomic dead space does not change significantly during pregnancy, at-term alveolar ventilation is increased by 70%. After the fifth month of pregnancy, the functional residual capacity, expiratory reserve volume and residual volume are all decreased by about 20% because of the gravid uterus pushing on the diaphragm. Vital capacity is not appreciably changed from prepregnancy lev-

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From the Department of Anesthesiology, Mount Sinai School of Medicine, New York, NY.

Address correspondence to Yaakov Beilin, M.D., Assistant Professor of Anesthesiology, Mount Sinai School of Medicine, Box 1010, One East 100th Street, New York, NY 10029.

TABLE 1

Physiologic Changes of Pregnancy

Respiratory:	
Minute ventilation	Increases by 50%
Tidal volume	Increases by 40%
Respiratory rate	Increases by 10%
Oxygen consumption	Increases by 20%
P_AO_2	Increases by 10 mm Hg
Dead space	No change
Alveolar ventilation	Increases by 70%
P_ACO_2	Decreases by 10 mm Hg
Arterial Blood pH	No change
Serum HCO_3^-	Decreases by 4 mEq/L
Functional residual capacity	Decreases by 20%
Expiratory reserve volume	Decreases by 20%
Residual volume	Decreases by 20%
Vital capacity	No change
Cardiovascular:	
Cardiac output	Increases by 30–40%
Heart rate	Increases by 15%
Stroke volume	Increases by 30%
Total peripheral resistance	Decreases by 15%
Femoral venous pressure	Increases by 15%
Central venous pressure	No change
Systolic blood pressure	Decreases by 0–15%
Diastolic blood pressure	Decreases by 10–20%
Intravascular volume	Increases by 35%
Plasma volume	Increases by 45%
Red blood cell volume	Increases by 20%
Gastrointestinal:	
Motility	Decreases
Stomach position	More cephalad and horizontal
Transaminases	Increases
Alkaline phosphatase	Increases
Pseudocholinesterase	Decreases by 20%
Hematologic:	
Hemoglobin	Decreases
Coagulation factors	Increases
Platelet count	Decreases by 20%
Lymphocyte function	Decreases
Renal:	
Renal blood flow	Increases
Glomerular filtration rate	Increases
Serum creatinine and BUN	Decreases
Creatinine clearance	Increases
Glucosuria	1–10 g/day
Proteinuria	300 mg/day
Nervous system:	
Minimal alveolar concentration	Decreases by 40%
Endorphin levels	Increases

Source: Beilin Y. Nonobstetric Surgery in the Pregnant Patient. In: Reed AP, editor. Clinical Cases in Anesthesia, 2nd ed. New York: Churchill Livingstone; 1995. p. 282. Reprinted with permission.

els. The increase in minute ventilation leads to a decrease in P_ACO_2 to approximately 30 mm Hg. Arterial blood pH remains unchanged because of a compensatory increase in renal excretion of bicarbonate ions.

Increased alveolar ventilation and decreased functional residual capacity lead to a more rapid uptake of inhaled anesthetics. The decrease in functional residual capacity, in conjunction with increases in cardiac output, metabolic rate, and oxygen consumption, makes the pregnant patient more susceptible to arterial hypoxemia during periods of apnea.

Edema, weight gain and increase in breast size may make intubation of the trachea technically difficult. An array of laryngoscope blades and handles, and other emergency airway management equipment should be available. Capillary engorgement of the mucosal lining of the upper airway accompanies pregnancy. This mandates extreme care during manipulation of the airway and the use of a smaller-than-normal tracheal tube. The use of a nasal airway and naso-tracheal intubation should be avoided.

Cardiovascular System

Cardiac output is increased by 30-40% during the first trimester. Further slight increases occur during the second trimester and last throughout pregnancy (7).

Blood pressure normally decreases during pregnancy because of a 15% decrease in systemic vascular resistance. Near term, 10–15% of patients have a further reduction in blood pressure in the supine position, often associated with diaphoresis, nausea, vomiting, pallor and changes in cerebration. This is the supine hypotensive syndrome and is caused by compression of the inferior vena cava by the gravid uterus. The symptoms of this syndrome can be alleviated by displacing the uterus by tilting the patient on her left side.

Intravascular volume is increased by 35% during pregnancy. Because plasma volume increases by a greater percentage than red blood cell volume (45% and 20% respectively), there is a relative anemia during pregnancy. Nevertheless, a hemoglobin of less than 11 g/dL is considered abnormal.

Increases in cardiac output will hasten the speed of intravenous inductions. Compression of the inferior vena cava by the gravid uterus leads to dilatation of the azygous system and the epidural veins. Epidural venous engorgement decreases the size of the epidural and intrathecal spaces, which explains why the doses of drugs used during a major conduction block must be decreased.

Gastrointestinal System

Due to increased progesterone levels, gastrointestinal tract motility is decreased by the end of the first trimester. The stomach, displaced upward by the enlarging uterus, eventually assumes a horizontal position, further slowing stomach emptying and changing the position and function of the gastroesophageal sphincter. Anxiety, pain and administration of opiates and anticholinergics will further slow gastric emptying. Gastrin production is increased during pregnancy (because the placenta produces gastrin) leading to an increase in acid production.

In view of these changes in gastric function, the pregnant patient is at increased risk for aspiration of gastric contents, after the first trimester. A non-particulate antacid, H_2 receptor blocker and metoclopramide should be used to decrease the acidity and volume of the gastric content. All general anesthetics should be conducted with a rapid sequence induction, cricoid pressure, and tracheal intubation.

Hepatic Changes

The results of tests of liver function (serum glutamic-oxaloacetic transaminase, lactic dehydrogenase, alkaline phosphatase and cholesterol) are frequently elevated during pregnancy. These elevations do not necessarily indicate abnormal liver function. Pseudocholinesterase activity declines by as much as 20% during the first trimester, which can lead to a prolonged duration of action of succinylcholine. This is rarely a problem following a standard dose of succinylcholine. Prolonged activity of local anesthetics from pseudocholinesterase deficiency has not been a problem.

Nervous System

Since pregnancy enhances alveolar ventilation, the pregnant woman is at risk for anesthetic overdose. The doses of all anesthetic agents for general or regional anesthesia should be reduced to assure that the minimum alveolar concentration (MAC) is decreased by up to 40%. This phenomenon is related to a progesterone and endorphin effect.

Fetal Considerations

Teratogenic Drugs

A teratogen is a substance that produces an increase in the incidence of a particular defect in a fetus that cannot be attributed to chance. In order to produce a defect, the teratogen must be administered in a sufficient dose at a critical point in development. In humans, this critical point is during organogenesis, which extends from 15 days gestational age to approximately 60 days gestational age. However, the central nervous system does not fully develop until after birth. Therefore, the critical time for this system can extend through the entire gestation.

Three approaches have been utilized to study the effect on the fetus of anesthetic agents and/or anesthesia administered to the pregnant patient: (1) animal studies, (2) studies of operating room personnel chronically exposed to trace concentrations of inhaled anesthetics, and (3) studies of women who underwent surgery while pregnant.

The applicability of results of animal studies is limited because of species variations and the much larger doses of anesthetic agents used than those used clinically. Additionally, other factors such as hypercarbia, hypothermia and hypoxemia were either not measured or not controlled. Moreover, with the exception of local anesthetics, virtually every anesthetic agent has been found to be a teratogen in some animal study. In contrast, with the exception of cocaine and possibly diazepam, no anesthetic agent has been proven to be teratogenic in humans (Table 2).

Benzodiazepines have questionable teratogenicity in humans. Some investigators noted an association between diazepam ingestion in the first six weeks of pregnancy and cleft palate in the newborn (8, 9). Although this finding has been questioned by the results of a prospective study by Shiono and Mills (10), the use of diazepam and other benzodiazepines should be avoided during pregnancy (11).

Nitrous oxide is a known teratogen (12) in mammals and rapidly crosses the human placenta (13). It had been presumed that the teratogenicity of nitrous oxide in animals is related to its oxidation of vitamin B_{12}, which then cannot function as a cofactor for the enzyme methionine synthetase. Methionine synthetase is needed for the formation of thymidine, a subunit of DNA. There is now evidence that the effects in animals of nitrous oxide are not related to possible effects on DNA synthesis. Pretreatment of rats exposed to nitrous oxide with folinic acid, which bypasses the methionine synthetase step in DNA synthesis, does not prevent congenital abnormalities (14), and suppression of methionine synthetase occurs at low concentrations of nitrous oxide (15), concentrations found safe in animal studies (16). Despite these theoretical concerns, nitrous oxide has not been found to be associated with congenital abnormalities in humans (3).

A number of epidemiologic studies (17, 18) were performed to determine the health hazards

TABLE 2
Teratogenic Effects of Anesthetic Agents in Animals

Anesthetic agent	Teratogenic effect
Thiopental	Cleft lip and cardiomyopathies
Opioids	Inguinal hernia and CNS[1]
Local anesthetics	Cytotoxic in tissue culture
Cocaine	GI[2], genitourinary, and CNS
Muscle relaxants	Musculoskeletal deformities
Inhalational agents	Cleft palate and skeletal abnormalities
Nitrous oxide	Skeletal abnormalities
Benzodiazepines	Cardiac defects, spina bifida, and cleft lip

[1]CNS = central nervous system
[2]GI = gastrointestinal

to operating room personnel, with respect to birth defects and spontaneous abortions, from chronic exposure to anesthetic gases. Operating room personnel (anesthesiologists, nurses, etc.) received questionnaires in the mail designed to gather information about the extent of their exposure and reproductive outcome. All the studies found that operating room personnel had an increased risk of spontaneous abortions and congenital abnormalities. However, these studies were later criticized (19–21) for their lack of control groups, low response rates to questionnaires, recall bias, and statistical inaccuracies.

There have also been several retrospective studies (2, 3, 22, 23) of pregnant women who had undergone surgery, to find a correlation between anesthesia and surgery, and congenital defects, spontaneous abortions, and fetal demise. No study found an increase in congenital abnormalities, but they all found risks to the fetus due to spontaneous abortions, premature deliveries and intrauterine growth retardation. These risks could not be linked, in any study, to either the anesthetic agents or the anesthetic technique; in most cases, the surgery was performed under general anesthesia using nitrous oxide. The increased risk to the fetus may be due to the condition that necessitated surgery in the first place, with the highest rate in gynecologic procedures.

Avoidance of Intrauterine Fetal Asphyxia

Intrauterine fetal asphyxia is avoided by maintaining normal maternal P_AO_2, P_ACO_2 and uterine blood flow. Maternal hypoxemia will lead to fetal hypoxemia and finally fetal demise. General anesthesia is a particular risk to the pregnant woman because management of the airway can be difficult and the rate of hemoglobin oxygen desaturation is increased due to the decreased functional residual capacity and increased oxygen consumption. However, care must also be taken during a regional anesthetic, since a high segmental level of anesthesia during a major conduction block, a toxic local anesthetic reaction or oversedation can also lead to a hypoxic event. There is no known adverse effect on the fetus if the pregnant woman breathes 100% oxygen during the procedure.

Both maternal hypercapnia and hypocapnia can be detrimental to the fetus. Hypocapnia produced by excessive positive pressure ventilation may increase mean intrathoracic pressure, decrease venous return and lead to a fall in uterine blood flow. In addition, maternal alkalosis, as produced by hyperventilation, will decrease uterine blood flow by direct vasoconstriction and it will decrease oxygen delivery by shifting the maternal oxyhemoglobin dissociation curve to the left. Hypercapnia is detrimental because it is associated with fetal acidosis and myocardial depression.

Uterine blood flow is affected both by drugs and anesthetic procedures. Placental blood flow is directly proportional to the net perfusion pressure across the intervillous space and inversely proportional to the resistance. Perfusion pressure will be decreased by hypotension that may be associated with epidural, spinal, or general anesthesia, from aorto-caval compression in the supine position, or from hemorrhage. Vasoconstriction following the use of α-adrenergic drugs will increase vascular resistance and decrease uteroplacental blood flow. A similar vasoconstrictor response may occur with decreased P_ACO_2 and increased catecholamine levels brought on by pain, apprehension, and light anesthesia.

Prevention of Premature Labor

Spontaneous abortions, premature labor and preterm delivery are the most significant risks to the fetus (3, 22). Mazze and Kallen (23) found that the risk of preterm delivery and subsequent fetal demise, in women who underwent appendectomy after the 23rd week of pregnancy, continued for one week after the surgery. Medications that have α-agonist properties (e.g., ketamine and phenylephrine) can increase uterine vascular tone and should be avoided. The potent inhaled anesthetic agents may be of some benefit by decreasing uterine tone and inhibiting uterine contraction. No study, however, has ever documented that any particular anesthetic agent or technique is associated with a higher or lower incidence of preterm labor. The highest risk is during gynecologic procedures when there is uterine manipulation and the lowest risk occurs during the second trimester.

General Recommendations

Whenever possible, anesthesia and surgery should be avoided during the first trimester. Precautions against aspiration should be taken from as early as the 12th week, and a clear non-particulate oral antacid, H_2 receptor blocker and metoclopramide should be administered. Ranitidine (50 mg i.v., given approximately 60 minutes prior to general anesthesia) has been shown to reduce the gastric pH in women undergoing cesarean section (24), and metoclopramide (10 mg i.v., given 15–30 minutes prior to general anesthesia) has been shown to be effective in reducing gastric volume in early pregnancy (25). Ranitidine and cimetidine have been shown not to be teratogenic in humans (26). This author is not aware of any human studies concerning the teratogenicity of metoclopramide in humans.

Apprehension should be allayed by personal reassurance, if possible, rather than with premedication. The patient can be reassured that there is no known increased risk to the baby regarding congenital malformations, but cautioned that there is an increased risk of premature labor and delivery. The patient should be transported to the operating room with left uterine displacement, to avoid the maternal supine hypotensive syndrome.

In addition to the routine intraoperative monitors, the fetal heart rate and uterine tone should be monitored, if at all possible. Using a Doppler apparatus, fetal heart rate monitoring becomes feasible after the 16th week of pregnancy. An external tocodynamometer can be used if the uterus is at or above the level of the umbilicus. These monitors may be technically difficult or impossible to use during an intra-abdominal procedure or in an obese patient. It is important that an obstetrician, or someone proficient in fetal monitoring, be present throughout the case to interpret the uterine/fetal monitoring. Also, there must be a plan as to how to proceed in the event of fetal distress. Prior to 23 or 24 weeks gestation, optimization of the maternal condition may improve the fetal condition. Katz et al. (27) reported a case in which the abnormal heart rate of a fetus was corrected by increasing the percentage of inspired oxygen given to the patient undergoing eye surgery. After 23-24 weeks gestation, in addition to attempts at correcting the intrauterine milieu, emergent cesarean section should be part of the plan. Monitoring of fetal heart rate and uterine tone monitoring should continue into the postoperative period (23).

The type of anesthesia should be based on maternal indications, the site and nature of the surgery, and the anesthesiologist's experience. Unless contraindicated, local or regional anesthesia may be preferable to general anesthesia, to avoid the risk of aspiration and decrease drug exposure.

If a spinal or an epidural anesthetic is to be used, then adequate prehydration (at least one liter of crystalloid) should be administered to prevent hypotension. If hypotension occurs, it must immediately be treated with the administration of more crystalloid or by using a drug such as ephedrine, which has predominantly beta adrenergic effects.

General anesthesia should be preceded by careful evaluation of the airway, preoxygenation, cricoid pressure and a rapid sequence induction. Preoxygenation is especially critical for the pregnant patient, in order to maximize the oxygen tension within the functional residual capacity of the lungs. This increase in oxygen tension allows the anesthesiologist more time to intubate the trachea prior to the onset of hemoglobin desaturation. An array of laryngoscopes, endotracheal tubes and other emergency airway equipment should be available. Although there are no data to support the use of one agent over another, drugs with a long history of safety in the pregnant patient should be employed. These include thiopental, morphine, meperidine, and fentanyl. The use of nitrous oxide during pregnancy is controversial and should be avoided if possible, especially during the first trimester. All the currently available muscle relaxants are safe to use during pregnancy, because they are highly ionized and thus undergo minimal placental transfer.

A high concentration of oxygen should be used (at least 50%), and arterial PCO_2 should be maintained within the normal range of 30–35 mm Hg for pregnancy. End-tidal CO_2 is an excellent approximation of P_ACO_2 in the pregnant patient because the arterial-to-end-tidal CO_2 gradient decreases during pregnancy (28).

Special techniques, cardiopulmonary bypass, hypothermia and hypotensive techniques have all been performed safely during pregnancy and should not be withheld (4, 5).

Fetal heart rate and uterine activity monitoring should continue postoperatively. Epidural or intrathecal opioids are an excellent choice for postoperative pain management because they usually cause minimal sedation, and lower doses can be utilized compared to their administration via intramuscular or intravenous routes. Non-steroidal anti-inflammatory drugs should be avoided, because they may cause premature closure of the ductus arteriosus (29). Also to be avoided are hypotension, hypoxemia, hypercarbia, hypocarbia, and hypothermia. Regardless of the technique, maintenance of a normal intrauterine physiologic milieu throughout the perioperative period is essential to assure a successful outcome.

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Concepts of Preemptive Analgesia for Postoperative Pain

Medhat R. Wassef, M.D.

Abstract

Background: The predictable relationship between surgical injury responses and the ensuing postoperative pain has led to the development of the concept of preemptive analgesia, with its potential to improve the quality of the postoperative period.
Methods: A review of publications concerning the studies of the physiology of pain and the development of clinical strategies toward the prevention of postoperative pain was undertaken.
Results: Clinical studies using various methodologies indicate that preemptive analgesia may, in some circumstances, attenuate postoperative pain. Unfortunately, these studies were not always reproducible. Preemptive analgesia in these studies failed to prevent or attenuate postoperative pain.
Conclusion: Techniques directed toward reducing and/or eliminating postoperative pain are still being developed, and their clinical utility is yet to be fully evaluated. **Key Words:** Preemptive analgesia, postoperative pain.

Introduction

THE CONCEPT OF PREEMPTIVE ANALGESIA derived from observations made in basic science and animal research, in which analgesics and/or local anesthetics were administered prior to surgery. The challenge remains to identify and evaluate clinically applicable methodologies appropriate to particular surgical procedures.

This paper reviews the concept of preemptive analgesia and some clinical studies which evaluate the impact of preemptive analgesia on pain during the immediate postoperative period.

The Development of the Concept

The concept of preemptive analgesia is based on prevention of central sensitization resulting from transmission of sensory nociceptive stimuli emanating as a result of surgery (1). The underlying principle requires that appropriate pharmacologic intervention be made well ahead of time of the onset of pain rather than in response to it. The aim of such treatment is to prevent the spinal cord from reaching a hyperexcitable state that leads to excessive response to afferent pain nerve impulses. Since the inflammatory reaction to tissue injury during surgery may serve as a source of the afferent nerve traffic postoperatively, therapy limited to the pre- and intraoperative periods alone may be insufficient. There is both scientific and clinical interest in preemptive analgesia and its potential for improving postoperative pain management. Wall (2) proposed that "preemptive preoperative analgesia" may have prolonged effects that long outlast the presence of drugs.

The finding that sensory signals generated from tissue injured during surgery can trigger a prolonged state of increased excitability of the central nervous system (3) has led to clinical studies to test whether preoperative local, regional anesthesia or opioid premedication can preempt postoperative pain.

The following three reports (4–6) imply that prevention may be possible. In one study (4), 18 multiparous women who had undergone elective cesarean section were assigned randomly to

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From the Department of Anesthesiology, Mount Sinai School of Medicine and Elmhurst Hospital Center, 79-01 Broadway, Elmhurst, NY 11373. Address correspondence to Dr. Medhat R. Wassef, Assistant Professor of Anesthesiology, Elmhurst Hospital Center, Department of Anesthesiology, E2-69, 79-01 Broadway, Elmhurst, NY 11373.

receive either anesthesia or epidural blocks. One to three days after the operation, the patients who had received epidural block complained of significantly less pain than those who had had general anesthesia. This difference is probably not due to the extent of surgery, but rather to the technique by which the anesthesia was administered.

The second study (5) reported the results of a prospective randomized survey of 929 patients undergoing orthopedic surgery under general anesthesia, in which the duration of postoperative pain relief provided by two prophylactic measures, either opiate premedication or nerve blocks with local anesthetic, was measured. The median time to first request for postoperative analgesia was 105 min (95% CI 100–115 min-$p<0.001$) when neither opiate premedication nor block was used. Those patients receiving opiate premedication increased this interval significantly. The median time was 328 min (95% CI 285–375 min-$p<0.005$). Local anesthesia block alone produced a further significant increase to 480 min (95% CI 415–585 min-$p<0.005$). Opiate premedication used with local anesthetic block extended the median time to more than 9 hours (95% CI 455–725 min-$p<0.005$).

In the third report (6), a total of 25 patients who underwent elective leg amputation were studied prospectively. Eleven patients were maintained pain-free for 3 days prior to their amputation with a lumbar epidural block using bupivacaine and/or morphine. All operations were performed under spinal or epidural anesthesia. All patients were interviewed about their limb pain before limb amputation, and about their phantom limb pain 7 days, 6 months, and one year after limb loss. Seven days after operation, 3 patients (27%) in the blockade group and 9 patients (64%) in the control group had phantom limb pain. After 6 months, of the 10 surviving patients in the blockade group, all were pain-free, in contrast to 5 of the 13 surviving patients (38%) in the control group. After one year, all 8 patients in the blockade group who were still alive were pain-free. However, 3 patients (27%) of the 11 still alive in the control group had phantom pain. The authors suggested that preoperative lumbar epidural blockade may reduce the incidence of phantom pain in the first year after amputation (6), but recommend further trials with patients undergoing lower limb amputation.

Rationale for Application of the Concept

The modern practice of general anesthesia entails the balanced use of small doses of general anesthetics and muscle relaxants. In this situation, the spinal cord receives continuous and heavy afferent nerve traffic consequent to surgical trauma, particularly when peripheral nerves are sectioned. It has been shown, in the decerebrate spinal rat, that the arrival of a volley of nerve impulses in unmyelinated C-fibers sets off a prolonged, widespread increase of reflex excitability (3, 7) and interneuron excitability (8). This triggered central hyperexcitability is particularly strong and prolonged if the stimulation is applied to deep tissues such as muscles or joint (7, 9). When peripheral nerves are cut, the subsequent brief but maximal injury discharge triggers prolonged spinal cord hyperexcitability (9) and even postsynaptic spinal cord morphological changes (10). It is thought that large doses of narcotics would be needed to suppress this hyperexcitable state (11). On the other hand, under regional anesthesia, these afferent signals to the spinal cord are blocked. Since a similar advantage in preventing the bombardment of the central nervous system with these afferent impulses may be possible with appropriate techniques alone or in combination with general anesthesia, minimal doses of narcotics given before any afferent barrage arrives may inhibit the triggered central hyperexcitability. Whether this approach provides a rationale for preoperative treatment designed to inhibit spinal cord hyperexcitability and limit excessive and prolonged responsiveness is yet to be fully evaluated.

Neurophysiological Background

Many advances in the management of pain over the past three decades derive from the gate-control theory by Melzack and Wall (12). They concluded that pain is not due to neural activity that resides exclusively in nociceptive pathways but is a result of activity in several interacting neural systems, each with its own specialized function. Obvious examples are "stimulation analgesia," such as the use of transcutaneous electrical nerve stimulation (TENS) (13, 14) and the use of "spinal opioids" (15). The papers of Wall (2) and Woolf (16) have stimulated much discussion on the potential clinical implications of this knowledge, from which the concept of "preemptive analgesia" evolved with respect to the management of acute postoperative pain (17).

Physiologic pain which results from ordinary everyday noxious stimuli is qualitatively different from the clinical pain experienced after tissue or nerve injury. The fundamental role of physiologic pain is to function as a protective and warning system against injurious stimuli. It is characterized by being well localized and transient, and having a high threshold. Moreover, its stimulus-response relationship is similar to that of other somatic sensory modalities (18, 19).

Clinical pain differs fundamentally from physiologic pain by the presence of pathologic hypersensitivity (20). It can be divided into inflammatory and neuropathic pain. The former refers to pain associated with tissue damage, such as that produced during surgery. The latter refers to damage to the nervous system. Tissue injury results in disruption of the normal specialization of the central nervous system, with alterations in the central processing mechanism of afferent stimuli (2, 16). Consequently, innocuous inputs from stimuli which would never produce pain may generate pain (allodynia). There also is increased pain (hyperalgesia) from suprathreshold stimulation, with development and spread of spontaneous pain (16, 21) and hypersensitivity to noninjured tissue (secondary hyperalgesia).

In response to surgical injury, the nervous system exhibits two states of activity. It was shown that if nociceptive information is allowed to reach the spinal cord, a state of peripheral (22) as well as central (1, 23) sensitization develops. The first is a state of peripheral sensitization that implies a reduction in the threshold of nociceptor afferent peripheral nerve ends. The second is a state of central sensitization that implies an activity-dependent increase in the excitability of the spinal cord neurons. Both states contribute to the post-injury pain hypersensitivity state found postoperatively. This is manifested by an increase in the response to noxious stimuli and a decrease in the pain threshold, both at the site of the injury and in the surrounding uninjured tissue.

Peripheral Sensitization

Peripheral sensitization to pain found with inflammation is produced by an increase in the sensitivity of the transduction mechanism in the high threshold nociceptors, the A-delta and C neurons, when inflammatory mediators such as norepinephrine, bradykinin, 5-HT, and prostaglandins are liberated as a result of tissue damage. Peripheral sensitization alters the high threshold in thermal and mechanoreceptors in the immediate vicinity of tissue injury. In humans, cutaneous injury is followed by alterations in thermal and mechanical sensibility (24–26). "Primary hyperalgesia" refers to changes within the area of injury and "secondary hyperalgesia" refers to changes in the undamaged tissue surrounding the injury. Primary hyperalgesia is explained by sensitization of peripheral nociceptors (24-26). Reduction of peripheral sensitization has been assumed to be the major role of NSAIDs through the inhibition of prostaglandin production.

Central Sensitization and Neuronal Plasticity

A change in the excitability of spinal cord neurons may trigger nociceptive afferent inputs. The properties of dorsal horn neurons and their receptive fields are not fixed but can change, a phenomenon known as plasticity. Changes in spatial, temporal and neuronal threshold occur with the post-injury hypersensitivity (20). Experimental studies have demonstrated that peripheral injury may result in expansion of receptive fields along with a decrease of the threshold of dorsal horn neurons (2, 16, 27, 28). This facilitation may be prolonged, depending on the stimulus and the type of afferents activated (2, 21).

The afferent barrage that initiates the central nervous system hyperexcitability is mediated by C-fibers, whereas the afferent input from the area of secondary hyperalgesia evoked by mechanical stimulation is believed to be mediated by A-fibers. Studies confirming spinal sensitization after nociception in human volunteers have been performed (29), but formal clinical studies and long-term implications of such changes for outcome after surgery are still lacking. In a randomized study (29), subjects received 4 mg of morphine administered via an epidural catheter through the L2-L3 interspace. The administration of epidural morphine, either prior to or after tissue injury, significantly increased pain tolerance threshold within the area of injury (area of primary hyperalgesia) and decreased the area of allodynia for brush and pin prick surrounding the injury (area of secondary hyperalgesia). It was suggested (29, 30) that the major effect of epidural morphine on secondary hyperalgesia is inhibition of C-fiber-mediated activity which maintains the altered response properties of central neurons responsible for secondary hyperalgesia.

Painful stimuli can produce supraspinal antinociception in addition to segmental spinal cord sensitization. Supraspinal descending neural systems significantly influence the selection, modulation and control of ascending sensory information by acting on synaptic transmission in the dorsal horn of the spinal cord (31). Modulation of nociceptive information occurs through inhibitory and facilitatory effects on spinal nociceptive neurons in the dorsal horn of the spinal cord. Major neurotransmitters, derived from descending inhibitory neural systems, and their receptors include the endogenous opioids, gamma aminobutyric acid (GABA) and the noradrenergic alpha$_2$ adrenoceptors. Stress-induced analgesia (SIA) phenomenon operates through the enhancement of release of these mediators. Recently, a randomized study of patients undergoing back surgery (32) found that surgical pain, in addition to the spinal (segmental) sensitization, was also followed by supraspinal antinociception (inhibition). Preemptive fentanyl administration decreased the former and augmented the latter (32).

The fundamental difference between peripheral and central sensitization is that the former enables low-intensity stimuli to produce pain by activating sensitized A-delta and C nociceptors, whereas the latter represents an input in normal low-threshold A-beta sensory neurons producing pain as a result of changes in sensory processing in the spinal cord (20).

Based upon experimental and clinical studies, it has been proposed that surgical trauma in humans may lead to comparable alterations in sensory processing, resulting in amplification and prolongation of postoperative pain, and possibly persistent postsurgical pain (2, 21). Four studies have investigated the effect of surgery on pain sensitivity in humans (33-36). Postoperative sensory thresholds were reported to be unaffected (34, 35) or decreased (36). Increased sensitivity and pain to noxious electrical stimulation in the A-beta range were observed in patients after gynecological surgery (33). Furthermore, the threshold of the nociceptive flexion reflex was decreased in the early (48 hours) postoperative phase (37).

Allodynia and hyperalgesia in postoperative patients may be a result of sensitization of central neurons. Thus, data demonstrating neuroplasticity in postoperative patients are limited and the relative contribution of the central sensitization to the intensity and duration of acute pain in humans has not been determined. No information is available on the degree or duration of noxious inputs required to induce central changes or whether postoperative pain itself may lead to alterations in dorsal horn excitability independent of the afferent barrage during surgery. Moreover, it is not known if the intensity and maintenance of postoperative pain are more dependent on central hyperexcitability than on continuous afferent barrage from the wound.

Clinical Studies and Their Outcome

A fairly large number of comparative studies of various approaches have been evaluated for the potential clinical value of preemptive analgesia. Three different types of clinical trials can be discerned (38). The first has been a comparison between a particular preoperative treatment in one group of patients with another group given no treatment. The second has consisted of trials comparing the efficacy of a particular treatment if given pre- or postoperatively. The third has involved a continuous administration of analgesics before, during and after the operation. The analgesic drugs which were used included local anesthetics, opioids, and NSAIDs. They were administered locally, epidurally, intrathecally, or systematically, either alone or in combination. To assess the efficacy of the different treatment regimens, investigators have used, most commonly, a visual analog scale (VAS) to rate the severity of pain, the time to first request for an analgesic, and the total amount of drug received during the postoperative period.

However, there are few clinical trials which specifically examined whether preemptive analgesia actually reduces the extent and duration of postoperative pain, and thereby improves patient satisfaction. Some studies showed lack of consistent results. Factors responsible included problems with the accepted definition of preemptive analgesia (39). Changes in sensory processing could alter standards of clinical pain measurements. Pooling data from various studies (meta-analysis) was suggested to combat the statistical lack of power found in some studies (40).

Preoperative Wound Infiltration or Regional Anesthesia Compared with No Treatment

Clinical reports of pre-incisional local anesthetic infiltration or nerve blocks performed under general anesthesia have been found for a variety of different operations such as tonsillectomy (41), inguinal hernia repair (42), cholecystectomy (43) and laparoscopy (44).

A study in patients ranging in age from 6 to 18 years demonstrated that 3 ml of 0.25% bupivacaine per tonsil given prior to the surgical incision significantly reduced the constant pain score for 6 days compared with a saline control group (41). Pain intensity on swallowing was also reduced significantly by the preoperative block for up to 10 days after the operation. Both groups received paracetamol in the same doses for pain. Surgery was performed by the same surgeon and the pain assessor was blinded.

In a prospective, randomized, double-blind study, pre-incisional local anesthetic infiltration combined with general anesthesia in patients undergoing inguinal herniorrhaphy was compared with general anesthesia alone and with spinal anesthesia (42). One surgeon performed a well-defined procedure. Subcutaneous and intramuscular infiltration of the abdominal wall with 0.25% bupivacaine 40 ml along the line of the proposed incision was made 5 min before the surgery. Pain at rest and mobilization and wound tenderness were assessed by an algometer. The results showed that pre-incisional block provided significant and prolonged analgesia for 24–48 hr after surgery, compared with general anesthesia alone.

In a randomized study of 30 patients undergoing cholecystectomy (43), pre-incisional intrapleural block using 0.5% bupivacaine 20 ml prior to general anesthesia was compared with general anesthesia alone. The degree of postoperative pain and the total dose of postoperative analgesic requirement were significantly reduced.

In a study of 80 patients undergoing day case laparoscopy (44), patients were allocated randomly to initial subdiaphragmatic administration of 0.25% bupivacaine 80 ml, 1% lidocaine 80 ml with epinephrine 1:200,000, saline 80 ml or nothing. Post-laparoscopic shoulder pain scores at rest were reduced significantly in the local anesthetic treatment groups compared with the non-treatment group for up to 48 hours after surgery.

In the previous studies, the pre-incisional local anesthetic infiltration or nerve block in patients undergoing surgery under general anesthesia resulted in less postoperative pain or analgesic requirement than no blocks. The analgesic effect of the pre-incisional regional block was found to outlast the predicted duration of the block. These positive results may support the concept of preemptive analgesia and may be explained by reduction of central hyperexcitability, but an effect on the peripheral nociceptors mediated by reduction of primary hyperalgesia from the anti-inflammatory effects of local anesthetic infiltration cannot be excluded (1, 42). However, the duration of the effect of maintaining a block of sensory inflow during surgery has not yet been adequately addressed. Also, the correlation between the duration of the resulting preemptive analgesia and the type and duration of the surgical stimulus, the extent of tissue damage produced, and the degree of conduction block of the surgical field, remains to be verified (23).

Preoperative Compared with Postoperative Wound Infiltration or Regional Anesthesia

Two randomized double-blind studies compared 1% lidocaine infiltration before or after surgery in patients undergoing inguinal herniorrhaphy under general anesthesia. In the first study (45), patients were randomly allocated to receive an identical inguinal field block with lidocaine either 15 min before surgery or immediately after surgery. No significant differences in these two groups were observed in the time to first request for morphine or total morphine consumption postoperatively. However, in the second study (46), the efficacy of pre-incisional and post-incisional wound infiltration with lidocaine was compared. The request for additional postoperative analgesics occurred significantly earlier by those who received post-incisional lidocaine infiltration than by those who received pre-incisional lidocaine.

In a third study, in which patients undergoing colonic surgery were randomized, an epidural dose of 0.75% bupivacaine 9 ml plus morphine 2 mg was administered either before or after surgery. Following surgery, both groups received low dose continuous epidural of 0.25% bupivacaine plus morphine 0.2% at a rate of 4 ml/hour. The results showed no significant differences in pain scores at rest or during mobilization, or in request for additional morphine (47).

In another randomized study, the dose of opiate required during the first 24 postoperative hours was reduced by 50% in those patients undergoing knee joint surgery in whom femoral nerve block with 20 ml of 0.5% bupivacaine was given before rather than after surgery (48).

Thus, these few studies suggest that there is no firm evidence that timing of analgesic treatment has important effects on postoperative pain or requirements for additional analgesics (1). However, it could be concluded that the pre-incisional wound infiltration is a more effective method of providing postoperative analgesia than post-incisional or postoperative infiltration. The positive findings from the previous studies also strongly support the pathophysiologic theory that prevention of hyperalgesia and/or central nervous system hyperexcitability, owing to the afferent barrage from the surgical area, is of major importance in reducing postoperative pain.

Peripheral and Central Blockade

In the multifaceted, prospective and randomized study mentioned above (42), spinal anesthesia using 0.5% bupivacaine 2.5 ml in patients undergoing inguinal herniorrhaphy was compared with pre-incisional local anesthetic combined with general anesthesia, and with general anesthesia alone. The results showed that preoperative incisional neural block combined with general anesthesia provided superior and prolonged analgesia for up to 48 hours after the operation, compared to general anesthesia alone. The spinal anesthesia group had intermediary analgesic effects. It was suggested that the less profound effect of the spinal anesthesia on the postoperative pain was due to a shorter duration of its effect (3.5 hours vs 9 hours with local infiltration). In addition, powerful nociceptive impulses are generated not only by the surgical procedure per se, but also by the action of proteolytic and inflammatory agents released into the wound tissues for several hours after surgery. Hence, a 9-hour effect with bupivacaine infiltration probably offered a better protection than spinal bupivacaine which lasted only 3.5 hours.

In another study, the effectiveness and duration of percutaneous ilioinguinal-iliohypogastric nerve block with 10 ml of 0.5% bupivacaine administered postoperatively was studied in patients undergoing herniorrhaphy under spinal anesthesia using 5% lidocaine. The nerve block combined with spinal anesthesia was found to reduce the postoperative pain significantly for 48 hours after herniorrhaphy compared with pain in patients operated under spinal anesthesia only (49). This study may support the concept that preoperative neural block may reduce late postoperative pain in small-scale surgery. However, the evaluation of another analgesic procedure administered after the operations was not studied (1).

No differences in pain were noted in three trials (47, 50, 51) when epidural or spinal blocks were used before or after surgery. At normal doses of epidural and spinal local anesthetics, both somatosensory-evoked cortical potentials and afferent-induced stress responses remain present (52). Therefore, neither short duration spinal nor epidural local anesthesia seems to be effective in preventing postoperative pain, even though each is effective for intraoperative analgesia and needs to be supplemented by pre-incisional regional infiltration or opioids (42, 46, 49).

The Use of Opioids

In a prospective nonrandomized study in patients undergoing surgery for lumbar disc prolapse and receiving pethidine (Demerol) or flurnitrazepam premedication (53), the use of opioids produced an increase in time to demand analgesia and reduced the number of patients requesting postoperative analgesia.

In a randomized double-blind study in patients undergoing thoracotomy, pre-incisional administration of a single dose of epidural fentanyl 4 μg/kg was found to be more effective than post-incisional administration in reducing pain intensity and analgesic requirement between 12 and 24 hours after thoracotomy (54). This result indicates a particular effectiveness of opiates in producing preemptive analgesia and supports the experimental findings that administration of opiates prior to the injury can prevent the development of central sensitization induced by surgery and later maintained by stimuli arising from the wound (55).

However, in a randomized, placebo-controlled, double-blind study of 19 patients undergoing trans-sternal thymectomy, preoperative epidural morphine 7 mg, when compared with saline, had no effect on postoperative pain scores or on the analgesic requirements beyond the initial 8 hour postoperative period (56).

In another randomized, placebo-controlled study, a preoperative bolus of alfentanil 30 μg/kg followed by intraoperative infusion of 40 μg/kg/hour had no effect on postoperative pain scores or requirement for additional opioids (57). The last two studies, therefore, do not support the experimental studies to suggest that administration of opioids prior to injury can reduce postoperative pain (1).

It can be concluded from all these studies that optimal dose, route, and timing of opioids to preempt postoperative pain require further investigation, but that their use might be advantageous, particularly when local anesthesia is technically not feasible.

The Use of NSAIDs

In a randomized study in patients undergoing laparoscopic surgery of less than 2.5 hours duration, the preoperative administration of ibuprofen provided longer-lasting analgesia than 75 μg of intravenous fentanyl administered just before termination of surgery (58). NSAIDs are usually thought to produce effects by decreasing peripheral sensitization and the activation of nociceptors. However, there is recent evidence that an analgesic action occurs centrally (22).

The Factor of Timing

Although initial studies of preemptive analgesia showed that preoperative blockade with local anesthetics or administration of opioids was more effective in reducing postoperative pain than control conditions involving no treatment, the results of subsequent investigations comparing the effects of preoperative treatment with the same treatment initiated after surgery have been inconsistent (20). Available data from the few studies comparing identical neural block techniques before and after surgery have not documented clinically relevant differences in postoperative analgesia or analgesic requirements. Several studies are available on preoperative neural block with local anesthetics compared with no treatment and most studies have found a reduction in late postoperative pain, request for additional analgesics, or both, compared with no neural block (41, 42, 44, 49). However, these studies do not demonstrate that such effects result from optimal timing of analgesia or a reduction of central hyperexcitability by preoperative analgesic treatment.

In experimental studies, acute pain behavior or hyperexcitability of dorsal horn neurons may be eliminated or reduced if the afferent barrage is prevented from reaching the central nervous system by a neural block with local anesthetics (59-61) or if the excitability of the central nervous system is suppressed with opioids before it receives a nociceptive input (55, 62). Similar antinociceptive procedures were less effective when applied post-injury (7, 10, 55, 59). However, one study showed spinal anesthesia administered 5 min after the injury also to be effective (60). No preemptive analgesia was found when alfentanyl was given before versus after skin incision for abdominal hysterectomy. The authors suggested a larger dose of alfentanyl might have shown a different effect (63). On the other hand, the timing of analgesia has been suggested to be of potential major importance in the treatment of postoperative pain (2, 21).

The difference between the effect of preemptive analgesia on post-injury nociceptive processing in experimental studies and on pain in clinical studies may have several explanations. Although central sensitization may contribute to postoperative pain in humans, surgical trauma differs from the conditioning stimuli applied in most experimental studies. Contrary to a well-localized thermal or chemical injury, or brief C-afferent fiber stimulation, the afferent input to the central nervous system during and after surgery is prolonged and extensive, with mixed cutaneous, muscular and visceral components. Central sensitization may be generated not only during surgery, but also in the postoperative period, because of persistent inflammation and hyperalgesia at the wound site. Thus intraoperative nociceptive block may not be adequate to reduce postoperative sensitization of central neurons. However, this difference was significant only for as long as the block was clinically effective. Furthermore, conventional analgesic methods (local anesthetics or opioids) may not provide total C-afferent fiber block during surgery.

Studies have been reported (45, 47) in which no differences were noted following identical preoperative or postoperative blocks. Similar observations also have been made when either preoperative epidural block-or no block was administered (64). Such outcomes have been thought to be due to insufficient afferent block. However, these studies document very clearly that clinically relevant and very effective blocks, even when administered before surgery, have no important effect on later postoperative pain during physical activity. They therefore do not suggest that preemptive analgesia, even when combined with relatively prolonged postoperative analgesia, prevents or reduces late postoperative pain. Several studies have documented that such blocks were ineffective in inhibiting potentials evoked by peripheral electrical stimulation (65–67) or inflammatory response to surgery (64, 68).

Therefore, further studies are needed on the optimal dose of analgesia and on the timing and duration of its administration to prevent alterations in dorsal horn neurons after more extensive tissue injury than that studied experimentally (7, 10, 59, 60, 69).

Conclusion

There has been interest in preemptive analgesia since Wall (2) raised the possibility that pain after surgery might be reduced by preventing intraoperative nociceptive impulses from reaching the spinal cord. The significance of this approach lies not only in the obvious immediate benefit of protecting the patient at the time of surgical trauma, but also in the possibility that such pretreatment will prevent the development of specific central neuronal changes which later amplify the peripheral signal and contribute to the heightened postoperative pain (6, 24). Both animal and clinical studies have provided evidence that regional anesthesia reduces the central component contributing to postsurgical acute pain. However, the role of opioids, administered centrally or systematically, has not been clearly defined (70).

Suffice it to say, the planning and management of pain relief should start preoperatively, continue throughout the operative procedure and be maintained postoperatively. Ideally, the patient should not experience intolerable pain. In other words, the anesthesiologist must aim to minimize and control acute pain and its accompanying somatic, autonomic, metabolic, hormonal and psychological responses. This effort will enhance early ambulation.

Woolf (16) concludes with two important clinical messages. First, he states that any attempt to obtain total analgesia, which blocks physiological as well as pathological pain, is inappropriate and that "the treatment of pain should not simply be the application of techniques that suppress sensation." The use of local and regional techniques can be very effective in modifying peripheral input, while afferent-induced excitability in the dorsal horn is best treated with opioids. Second, it is clear that prevention is better than relief, as Woolf (16) also states, " . . . the dose of morphine required to prevent C-fiber induced excitability changes from occurring in the spinal cord is an order of magnitude lower than that required to suppress these changes once they occur."

Although the concept of preemptive analgesia is attractive, more clinical trials are required to study its efficacy. The controlled administration of various combinations of analgesic agents that will be specifically and simultaneously targeted to work at every level of the peripheral and central nervous system involved in the pathophysiological response to surgical pain will be a major objective of the future practice of anesthesiology.

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Multiple Cerebral Infarction in Japanese Breath-Hold Divers:

Two Case Reports

Kiyotaka Kohshi, M.D.,[1,2] Yoshimasa Kinoshita, M.D.,[1]
Haruhiko Abe, M.D.,[3] and Toshio Okudera, M.D.[4]

Abstract

We report on two Japanese breath-hold divers (ama) who developed neurological disturbances following more than 3 hours of consecutive dives to 15 – 25 meters of seawater. Their magnetic resonance images of the brain showed multiple cerebral infarcts which were consistent with their neurological symptoms. The cerebral lesions seem to have been caused by repetitive breath-hold dives for extended periods of time. Immediate recompression is required when neurological symptoms develop after such dives. **Key Words:** Dysbaric accident, decompression sickness, breath-hold diving, Japanese male divers, cerebral infarction, magnetic resonance imaging, diving.

Introduction

Dysbaric diving accident is a syndrome which follows a rapid reduction in ambient pressure, such as occurs during or after inadequate decompression. It includes decompression sickness (DCS) and pulmonary barotrauma. Barotrauma involves tissue damage resulting from a change in volume of entrapped gas during decompression. Decompression sickness occurs when bubbles of inert gas (nitrogen, for divers breathing air) are formed in the tissues as the ambient pressure is lowered rapidly. Type 1 DCS is the less serious variety and is characterized by joint pain and rash. Severe cardiopulmonary involvement and the signs of neurological deficits are prevalent in type 2 DCS.

Dysbarism has been assumed to be rare in breath-hold divers because of the low permeability of alveolar nitrogen gas (1). However, Paulev (2) observed the occurrence of apparent DCS when he made 60 breath-hold dives to 15 – 20 meters in 5 hours. Moreover, he noted that a syndrome called "Taravana," which occurs in breath-hold divers of the Tuamotu Archipelago in French Polynesia, was regarded as type 2 DCS. Diving accidents, however, have not been reported in either Korean or Japanese professional breath-hold (ama) divers. Recently, we encountered 2 Japanese ama divers with neurological disturbances following repetitive dives. Magnetic resonance imaging (MRI) of the brain was used to verify cerebral lesions.

Case Reports

Patient 1. A 44-year-old, right-handed male diver started his profession at the age of 15 and continued his work until this diving incident. He was a nonsmoker and his physical and laboratory examinations (including triglyceride, rheumatoid factor, antinuclear antibodies, electro- and echocardiography) were normal. He was a par-

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From the Departments of [1]Neurosurgery and [2]Hyperbaric Medicine, [3]Second Department of Internal Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan, and [4]Department of Radiology, Akita Research Institute of Brain and Blood Vessels, Akita, Japan. Address correspondence to Dr. K. Kohshi, Departments of Neurosurgery and Hyperbaric Medicine, University of Occupational and Environmental Health, 1 - 1 Iseigaoka, Yahatanishi- ku, Kitakyushu 807 Japan.

tially assisted diver who descended to 15 – 25 meters of seawater with a 15-kg weight and ascended without assistance. He continued to make 1 minute dives with 1-3 minute surfacing intervals between successive dives. He generally dove in 2 shifts a day, one in the morning and one in the afternoon, taking a lunch break in between. When he continued to alternate diving and surfacing for more than 3 hours, he occasionally experienced euphoria and cloudy consciousness. His diving time became progressively longer without difficulty in breathing. Rarely, he experienced dizziness, double vision and nausea. On September 3, 1990, he began to dive at 9:30 AM, as usual, and continued repetitive dives without a break. At about 1:00 PM, he boarded his boat due to nausea and double vision. About 10 minutes later, he was unable to speak spontaneously and felt weakness and numbness of the right upper and lower extremities. Disturbed speech was transient and disappeared completely after 10-20 minutes, although hemiparesis and hemidysesthesia remained for 1 and 4 weeks, respectively. Two weeks after the accident, he was transferred to a local hospital where he was examined with computed tomographic (CT) and MRI scans of the brain. The CT scan showed no abnormalities, but the MRI scan revealed 2 small lesions (low signal intensity in T1- and high signal intensity in T2-weighted imaging) in the left internal capsule and the left frontal lobe (Fig. 1).

Patient 2. A 38-year-old male diver from the same village had routinely dived to depths of 15 – 25 meters of seawater over the preceding 20 years. His diving and surfacing times were similar to those of patient 1. He occasionally felt nausea when he made continued consecutive dives for more than 3 hours. On August 3, 1986, he began to dive at about 9:30 AM and continued repetitive dives for more than 4 hours without a break. He took a break to eat lunch, but about 10 minutes after boarding his boat, he suddenly complained of hemiparesis and sensory disturbance in the left upper and lower extremities. One hour later, he could grasp with his left hand and 2 hours later he was able to walk, but sensory disturbance remained. Three hours after the onset of the accident, he lost consciousness but recovered gradually several hours later. His sensory disturbance disappeared completely several days later. He came to the hospital for detailed examination 4 years after the incident. Neurologically, there were no abnormal findings. Tl-weighted MRI showed multiple low-signal intensity lesions in the subcortical and subependymal areas, which became high in signal intensity in T2-weighted imaging (Fig. 2). He was a smoker but he had no risk factors for stroke, such as hypertension, diabetes mellitus, or cardiac arrhythmia. Other physical and laboratory examinations were normal.

Discussion

The cerebral lesions demonstrated by MRI in our 2 cases corresponded to their presenting neurological symptoms. The lesions may have been caused by repetitive breath-hold dives in both cases, because the divers were young males without major risk factors for stroke. This is the first report of Japanese ama divers who had experienced episodes of neurological disturbances following repetitive breath-hold dives.

No diving incidents among Japanese or Korean ama divers have been reported in medical records for at least 2 decades (3). In the first patient's village of Utsu on Mishima Island, Yamaguchi Prefecture, 26 professional male breath-hold divers are engaged in daily diving work during harvesting season. We performed interviews in this village to determine whether other similar diving incidents had occurred in ama divers. This survey revealed that 8 of 15 divers had previously experienced some neurological disorders during or after dives. The pearl divers of Tuamotu Archipelago, referred to earlier, are reported to have developed "Taravana" diving syndrome after repetitive dives to depths of 30 meters or more during a 6-hour period (4). Their symptoms included vertigo, nausea, paralysis, unconsciousness, and even sudden death. Those with motor paralysis, the most common symptom, recovered entirely in almost all cases. In general, the characteristics of the diving incidents in both groups of divers were similar, but the severity of the incidents was greater for the Tuamoto group.

Although diving incidents have always been theoretically possible in ama divers, they have not been reported in Japan or Korea for some 25 years. Based on our observations, we surmise that neurological evaluations of the divers has not been carried out on a regular basis. On the other hand, Paulev (2) noted that, following consecutive breath-hold dives, his serious symptoms included central nervous system (CNS) involvement such as paralysis, visual disturbance and unconsciousness. He concluded that these symptoms were consistent with the diagnosis of decompression sickness (DCS) and that this dramatic response to recompression could not be explained by any causes other than the presence of bubbles in the CNS. The possibility thus exists

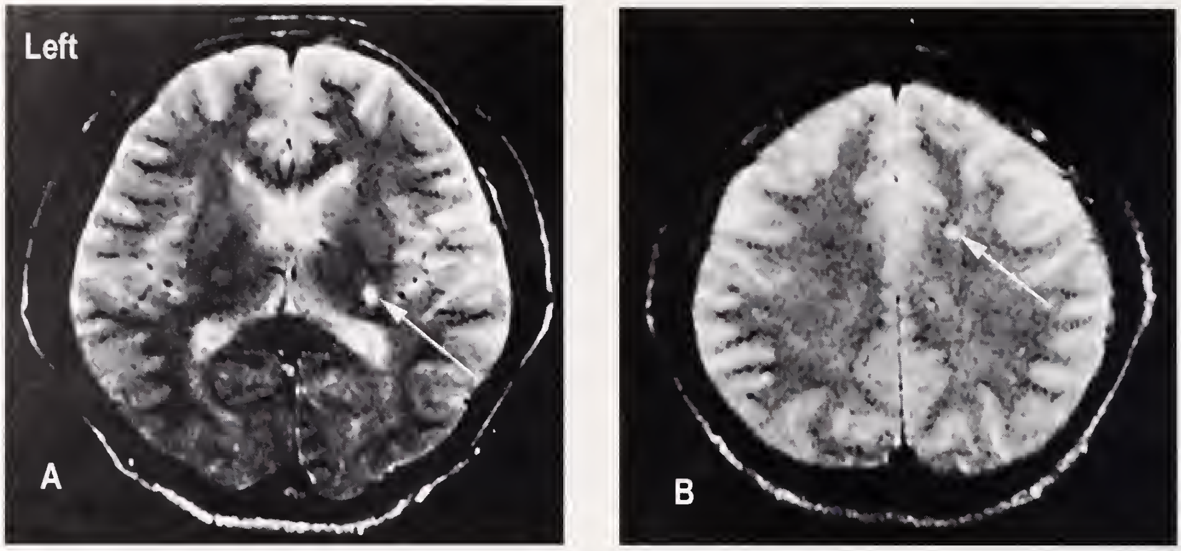

Fig. 1. MRI of the brain in patient 1. T2-weighted imaging (TR/TE: 2000/110) shows increased signal intensity in the left internal capsule and the left frontal lobe (arrows).

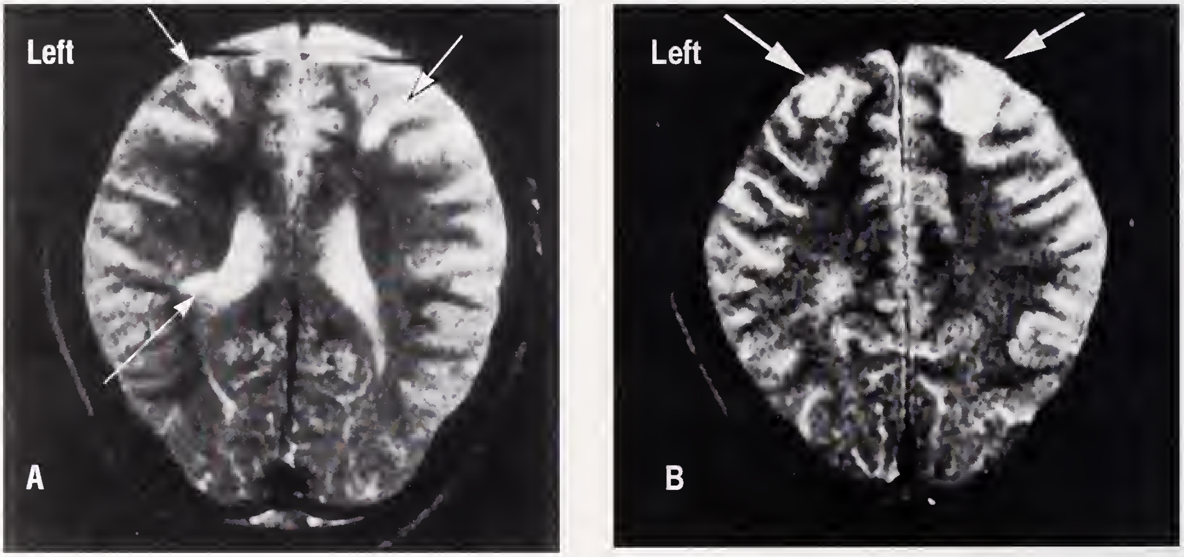

Fig. 2. MRI of the brain in patient 2. T2-weighted imaging (TR/TE: 2000/110) shows increased signal intensity in the bilateral frontal lobes and right periventricular region (arrows).

that long-term deep breath-hold dives induce CNS damage.

The mechanism for CNS involvement following repetitive breath-hold dives is poorly understood. The locations of the lesions demonstrated by MRI in the present cases are the terminal and border zones of the cerebral vasculature, where the arterial blood and oxygen supply are physiologically poor and the perfusion pressure is low (5). On MRI studies in compressed air divers, the CNS lesions caused by nitrogen gas bubbles are also similar to those in our cases (6–8). It is suggested that this type of cerebral lesion is due to vascular pathogenesis, especially arterial events. Some investigators have suggested the accumulation of nitrogen gas in peripheral tissues following repetitive breath-hold dives (2, 9, 10). Venous gas bubbles arise due to a continuous release of inert gas (nitrogen) from the peripheral tissues during and after decompression. In fact, Spencer and Okino (3) recorded formation of silent intravascular bubbles in a Japanese breath-hold diver after 30 consecutive dives to the depth of 15 meters of seawater over a period of 51 minutes. Microbubbles larger than 20 μm in diameter are completely filtered by mammalian lungs (11, 12).

It has been assumed that smaller bubbles released by the lungs are harmless, because there is evidence that they do not arrest in the cerebral circulation. Hills and James (13), however, have recently reported that the blood-brain barrier is impaired by such smaller bubbles. Since gas microbubbles in the arterial circulation are propelled by hydrostatic forces to reach the terminal and border zones (14), the cerebral lesions are likely due to microbubbles passing through the lungs. We propose that venous gas microemboli may enter the systemic circulation and cause arterial embolization. Some other possible mechanisms include severe hypoxia during ascent or the presence of patent foramen ovale (15, 16). The breath-hold divers should be treated immediately by recompression therapy when they have neurological symptoms during or after repetitive breath-hold dives.

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Severe Hyponatremia, Neuroleptic Malignant Syndrome, Rhabdomyolysis and Acute Renal Failure:

A Case Report

CRISTINA ELIZALDE-SCIAVOLINO, M.D., ALEX RACCO, M.D.,
TAMIE PROSCIA-LIETO, M.D., AND MORTON KLEINER, M.D.

Abstract

Acute renal failure secondary to myoglobinuria is a rare yet possible complication of malignant neuroleptic syndrome associated with the use of dopamine antagonists. We describe the case of a 42-year-old schizophrenic man who presented with severe hyponatremia, and proceeded to acute malignant neuroleptic syndrome, rhabdomyolysis, and acute renal failure. We contend that the acute hyponatremia may have served as a precipitating factor. **Key Words:** Hyponatremia, psychogenic polydipsia, inappropriate secretion of antidiuretic hormone, neuroleptic malignant syndrome, rhabdomyolysis, acute renal failure.

NEUROLEPTIC MALIGNANT SYNDROME (NMS) is a potentially fatal idiosyncratic reaction characterized by hyperthermia, mental status changes, muscle rigidity, diaphoresis, tachycardia, tachypnea, and autonomic dysfunction (1, 2). Although uncommon, it carries an incidence of 0.02–3.23% with the use of neuroleptic drugs (3). NMS lies within the spectrum of adverse reactions to dopamine antagonists, along with renal failure and rhabdomyolysis. We report on a patient who presented with severe hyponatremia, and progressed to NMS, myoglobinuria, and acute renal failure. While our literature search (4–6) reveals previous cases which implicated hyponatremia as a precipitating cause, other factors may play a role in the pathogenesis of this syndrome.

Case Report

A 42-year-old man with a history of paranoid-type schizophrenia was transferred to our medical facility for the evaluation of both confusion and emesis of one-half gallon of clear fluid. He was noted to have been drinking "gallons" of water in the previous days. His medications included buspirone hydrochloride and thiothixene. On transport to the facility, he experienced a generalized seizure that resolved spontaneously. Vital signs upon presentation to the emergency department showed a blood pressure of 180/90 mm Hg, temperature of 98.3°F, pulse of 130 beats per minute, and a respiratory rate of 32 breaths per minute. He was agitated and confused; however, his physical exam did not show any tremor, nuchal rigidity, weakness, papilledema, muscle rigidity, or cranial nerve disturbance. A second and final generalized seizure lasting 45 seconds was witnessed and treated with 2 mg lorazepam intravenously.

From the Department of Medicine, Staten Island University Hospital, Staten Island, NY. Address correspondence to Morton Kleiner, M.D., 470 Seaview Avenue, Staten Island, NY 10305.

Laboratory studies yielded the following results. Serum creatinine was 0.9 mg/dL (79.5 mM), and serum electrolytes showed a sodium of 114 mEq/L, potassium of 3.9 mEq/L, chloride of 84 mEq/L, bicarbonate of 12.5 mM, and blood urea nitrogen of 6 mg/dL (2.1 mM). Urinalysis was remarkable for a specific gravity of less than 1.005, sodium concentration of 47 mEq/L, potassium of 6.3 mEq/L, creatinine of <10 mg/dL, and osmolarity of 139 mOsm/kg. Hematological studies showed a white blood cell count of 21,000 mm^3 (21 x 10^9/L), hemoglobin of 13.7 g/dL (137 g/L), and a platelet count of 384,000/mm^3 (384 x 10^9/L). Intravenous infusion of 0.9% sodium chloride (154 mEq/L sodiumisotonic saline) was then started at a rate of 60 mL/hr.

A computerized tomographic scan of the head did not reveal the presence of any ischemic event or hemorrhage. A lumbar puncture revealed a white blood cell count of 12 cells/mm^3 with a differential count of 93% polymorphonucleocytes, no red blood cells, and concentrations of protein and glucose of 46 mg/dL (0.46 g/L), and 81 mg/dL (4.45 mM) respectively. Cultures of blood, urine and cerebrospinal fluid all remained negative. Intravenous cefotaxime was started empirically in response to the presence of leukocytes in the spinal fluid.

Subsequently, the patient's hyponatremia, which was felt to be secondary to psychogenic polydipsia, was corrected. Although there was no recurrence of seizures following the first two episodes, the fluctuating temperature spikes continued. During the first hospital day, his temperature rose to a maximum of 104.1°F. Figure 1 illustrates the temperature pattern curve. The fevers persisted despite the use of cooling blankets and acetaminophen at a dose of 650 mg every four hours. On the evening of the second day, he became lethargic and nonverbal, and developed generalized muscle rigidity. Urinalysis by dipstick was 3+ positive for hemoglobin, but red blood cells were not seen microscopically. Diagnosis of probable malignant neuroleptic syndrome was made, and a stat dose of 1 mg/kg dantrolene sodium was given intravenously. All neuroleptics were discontinued. An intravenous fluid challenge consisting of 500 mL of 0.9% sodium chloride was given over 30 minutes, followed by 80 mg of intravenous furosemide. An improvement in urine output was noted after this therapy, as the output increased from 35 mL/hr to 100 mL/hr within the next 90 minutes. Intravenous fluid was administered thereafter with 5% dextrose in 0.9% sodium chloride at 100 mL/hr for 2 hours; it was then increased to 200 mL/hr. The urine output following this increase was 1300 mL over the next 7 hours. The patient was then transferred to the intensive care unit with the diagnosis of acute renal failure with rhabdomyolysis.

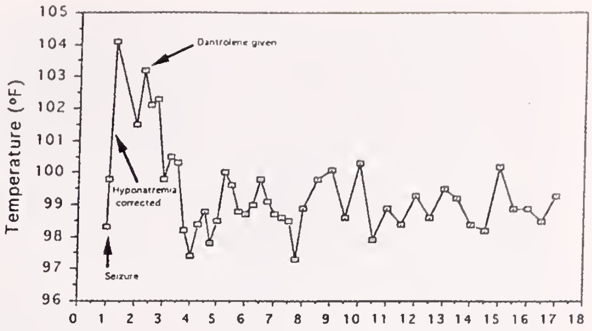

Fig. 1. Temperature pattern curve.

A serum creatine kinase drawn at this time revealed the level to be greater than 234,500 U/L (3909 mkat/L). Other laboratory results showed an increase in blood urea nitrogen to 16 mg/dL (5.7 mM), creatinine to 1.6 mg/dL (141 μM), and serum sodium and potassium of 135 mEq/L and 3.81 mEq/L respectively. Urinalysis revealed the presence of more than 300 mg/dL of protein, a trace of ketones, and positive test for hemoglobin and leukocytes. When examined under microscopy, numerous brown, granular, pigmented casts were noted, along with some red blood cells (Fig. 2). Urine myoglobin levels were not obtained at initial consultation.

By the following day, the patient's muscle rigidity and fever had resolved, and mental status was markedly improved. Although he remained non-oliguric throughout this episode, his blood urea nitrogen and serum creatinine peaked at 52 mg/dL (18.6 mM) and 6.3 mg/dL (557 mM) respectively by day 6 (Fig. 3). Urine myoglobin analysis from the fourth hospital day revealed a level of greater than 1585 μg/L in a volume of 3050 mL urine/24 hr. Dantrolene sodium was continued at 60 mg every 6 hours intravenously until day 7, when it was changed to 50 mg orally every 6 hours.

The patient's mental status continued to improve, but by day 10, signs of his underlying psychiatric disease became manifest. He was transferred back to the psychiatric facility on hospital day 18. At that time his blood urea nitrogen was 16 mg/dL (5.7 mM), and serum creatinine and sodium of 1.1 mg/dL (97.2 mM), and 137 mEq/L respectively.

Discussion

Neuroleptic malignant syndrome (NMS) is a complex and potentially fatal reaction to neu-

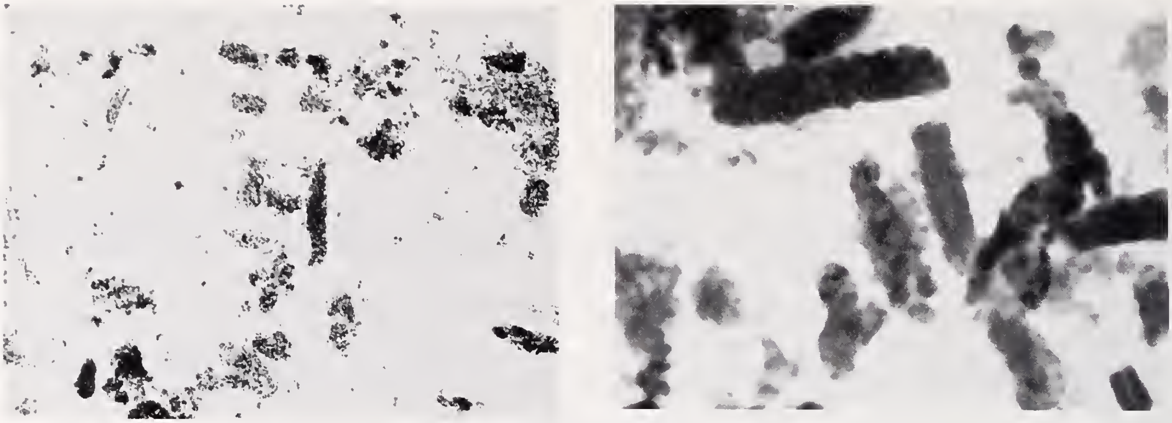

Fig. 2. Microscopic urine analysis under low power objective (on the left) and high power (on the right) depicting numerous brown, granular, pigmented casts and red blood cells.

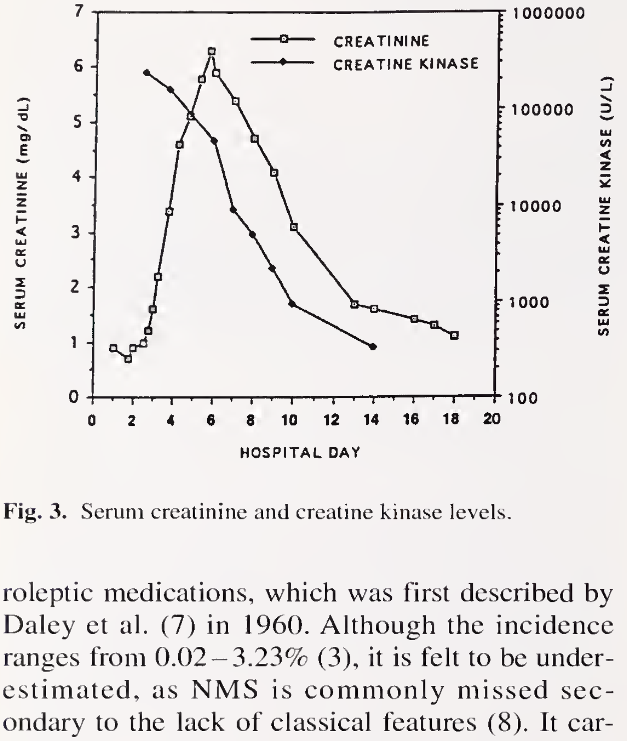

Fig. 3. Serum creatinine and creatine kinase levels.

roleptic medications, which was first described by Daley et al. (7) in 1960. Although the incidence ranges from 0.02–3.23% (3), it is felt to be underestimated, as NMS is commonly missed secondary to the lack of classical features (8). It carries with it a mortality rate in a range of 11–38%, largely due to cardiovascular collapse, acute renal failure, pulmonary embolism, aspiration pneumonia, and respiratory failure (8). Especially vulnerable are younger males less than 40 years of age (80% of patients) with a male:female ratio of 2 (9, 10). Gratz et al. (11) defined NMS by major criteria, including muscle rigidity, fever, and elevated creatine kinase, along with minor criteria of tachypnea, tachycardia, altered level of consciousness, abnormal blood pressure, diaphoresis and leukocytosis. Our patient fulfilled all three major, and four of the minor criteria.

Although the exact etiology of NMS is not known, D_2 dopamine receptor blockade produced by neuroleptics at multiple sites has been implicated (1, 2, 7, 9, 11, 12). Blockage of corticolimbic dopaminergic transmission may account for the mental status changes (9), whereas the hyperthermia and a hyperadrenergic state may occur as a result of blockage of hypothalamic dopaminergic tracts, which may also disrupt vasomotor thermoregulatory function (12). Dopamine antagonism may indirectly stimulate prostaglandin synthesis, which in turn might cause hyperthermia and enhance and derange calcium mobilization (4) and calcium homeostasis respectively. Dantrolene may act, in part, by reducing intracellular calcium ion activity by sequestering calcium within the sarcoplasmic reticulum, thus causing muscle relaxation (3). The accompanying metabolic acidosis exhibited by our patient may also have had an effect on calcium distribution.

Muscle rigidity is noted with striatal dopamine receptor blockage (12). Most consistently seen are significant elevations in serum creatine kinase, which reflects muscle rigidity and myonecrosis from sustained muscular contractions. Creatine kinase is a nonspecific finding that may be elevated with concurrent muscle damage caused by intramuscular injections, coma, seizures, hypothyroidism, and catatonia (11). Caution should be used when interpreting elevated creatine kinase levels, since one study (13) demonstrated significant elevation of creatine kinase levels in 70% of patients who became febrile while on psychotropics. The maximum creatine kinase level encountered in these patients was 2515 U/L (41 mkat/L). These patients were shown to have infections that mimicked the hyperthermia of NMS. Gurrera and Romero (14) added that LDH, SGOT (aspartate aminotransferase, AST), and serum aldolase correlate significantly with creatine kinase in NMS. Our patient's serum creatine kinase level of greater than 234,500 U/L (3909 mkat/L) on day 2, and urine myoglobin level of greater than 1585 µg/L on day 4 strongly support the diagnosis of rhabdomyolysis. Also, LDH and SGOT (AST) levels were both elevated at 1958 U/L (32.6 mkat/L) and 1607 U/L (26.8 mkat/L) respectively.

Myoglobinuria and renal failure have been associated with an increase in mortality to 50% (15). Myoglobinuria has also been noted to occur with immobilization or posturing catatonia. Marsh and Dolson (16) reported a case of rhabdomyolysis secondary to neuroleptic use that developed without signs of significant muscle rigidity or hyperthermia.

Various triggering factors for NMS have been proposed in the literature, e.g., ambient climate, neuroleptic dose changes, "prn" doses, infection, low iron concentrations, nutritional deficiencies, and the physiological state of the patient (11, 17, 18). Fujisawa et al. (19) reported a case of a contributing role of hyperreninemia with rhabdomyolysis secondary to NMS. Electrolyte imbalances, with or without dehydration, have also been suggested as a cause for NMS (17, 18). Acute hyponatremia has been hypothesized to exacerbate NMS, but the exact mechanism is not known (4–6).

While it has been proposed that psychosis itself may alter osmostat function (20), inappropriate secretion of antidiuretic hormone (SIADH), which may accompany psychogenic polydipsia (20), also has been suggested as a cause of NMS. SIADH has been attributed to treatment with psychoactive drugs, which may lead to "resetting" of the ADH osmostat. The term "osmostat" or "osmoreceptor" refers to the neuronal cells in the supraoptic nucleus in the anterior hypothalamus, which synthesize antidiuretic hormone (ADH) and initiate the antidiuretic response (21). Barlow and de Wardener (22) found that polydipsia did not, of itself, dilute body fluids appreciably because of the tremendous excretory capacity of the kidneys to excrete water when ADH secretion was suppressed. Failure to suppress ADH synthesis and release would predispose to the hazard of water intoxication and hyponatremia (20).

Our patient was known to have polydipsia and presented with hyponatremia while on neuroleptics. It is our feeling that he developed hyponatremia secondary to polydipsia and possible SIADH. Escrig et al. (23) found an association between SIADH and NMS which suggested a central pathogenic cause of NMS. Thiothixene has been noted to cause polydipsia, in addition to its action of dopaminergic blockade (24). Its use also may have played a role in this patient. Yamaguchi et al. (25) reported a case of NMS associated with SIADH-induced hyponatremia. Laboratory studies showed a transient increase in ADH secretion. They hypothesized that the hypothalamic disturbance in NMS may induce leakage of stored ADH from neuroendocrine neurons.

In summary, we report a case of NMS complicated by rhabdomyolysis and non-oliguric acute renal failure. We postulate that several factors, including the use of neuroleptic agents, led to SIADH, hyponatremia and the observed clinical sequence of events. Additional factors may have been related to possible alterations in calcium metabolism and metabolic acidosis.

Acknowledgments

The authors wish to thank Dr. Hooney Kahng for his microphotography, and Dr. Peter Sciavolino for his assistance with the figures. We also extend our gratefulness to the Staten Island University Hospital Library staff for their help with the literature search, and to Ms. Carolyn Scully for her secretarial assistance.

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Complications of Subcutaneous Injection Ports

Francis A. Dysarz, III, M.D., Michael A. Fiorillo, M.D.,
and Paul G. Davidson, M.D., F.A.C.S.

Abstract

Subcutaneous injection ports have become widely used for patients who require prolonged central venous access. Complications of their placement and use are well documented in the existing literature. We report two previously undocumented complications occurring in Port-a-Cath® units, and suggest methods to lessen the likelihood of recurrence of these problems. **Key Words:** Subcutaneous injection ports, vascular access, complications, Port-a-Cath®, Infuse-a-Port®.

Introduction

Since its introduction in 1982, the subcutaneous injection port (SIP) has gained wide acceptance. SIPs (Port-a-Cath® [SIMS Deltec Inc., St. Paul, MN], Infuse-a-Port® [Strato Infusaid/HMP, Manchester, GA], and others) have been shown to be reliable and effective devices for a broad range of patients who require prolonged central venous access (1 – 5). SIPs may be used for the administration of total parenteral nutrition, blood and its components, and chemotherapy, or for the withdrawal of the patient's blood for testing (6). As with any central venous access device, SIPs are subject to a variety of complications, both early and late.

Early complications, those occurring within the first two weeks of implantation, include pneumothorax, hemothorax, infection, bleeding, cervical neural plexus lesions, and incorrect catheter tip positioning (6, 7). Late complications, those occurring more than two weeks after implantation, include infection, clotting, extravasation, catheter embolization, local skin necrosis, and catheter tip migration (8, 9).

Eight instances of catheter tip migration from superior vena cava to internal jugular vein have been reported in the literature to date. This is the first paper in the literature to report complete extravascular migration of the catheter portion of a SIP (10). We also report a case in which a SIP flipped 180° within its subcutaneous pocket.

Case Reports

Patient 1: H.M., a 69-year-old white female diagnosed with bilateral invasive ductal breast carcinoma, underwent placement of a SIP for adjuvant chemotherapy (11). The device was placed into a left infraclavicular subcutaneous pocket and the catheter was positioned into the superior vena cava via the left subclavian vein using a percutaneous Seldinger technique. The operation was uneventful and no evidence of abnormal anatomy was encountered. Postoperative chest radiograph in the anteroposterior (AP) projection confirmed the position of the catheter tip in the superior vena cava (Fig. 1). The patient tolerated surgery well and was discharged from the hospital shortly after the procedure.

Approximately one month after its insertion, the SIP, which had been functioning well, became unusable for the infusion of medication and withdrawal of blood. An AP chest x-ray done shortly thereafter, as part of an unrelated dyspnea work-

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From the Department of Surgery, Staten Island University Hospital, Staten Island, NY. Address correspondence for reprints to Paul G. Davidson, M.D., F.A.C.S., 1130 Victory Boulevard, Staten Island, NY 10301.

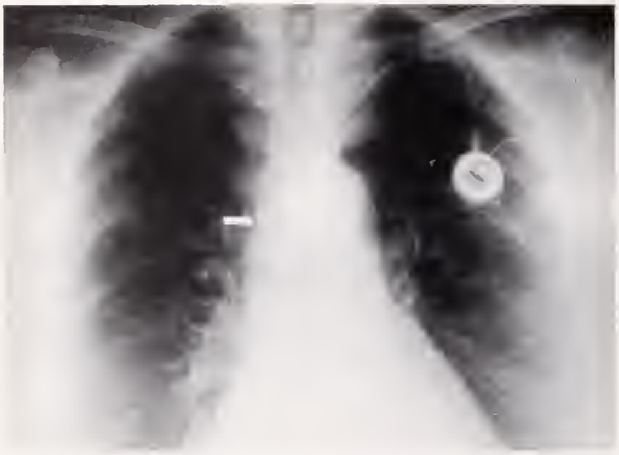

Fig. 1. The radiograph taken immediately after insertion shows the catheter tip (arrow) properly positioned in the superior vena cava.

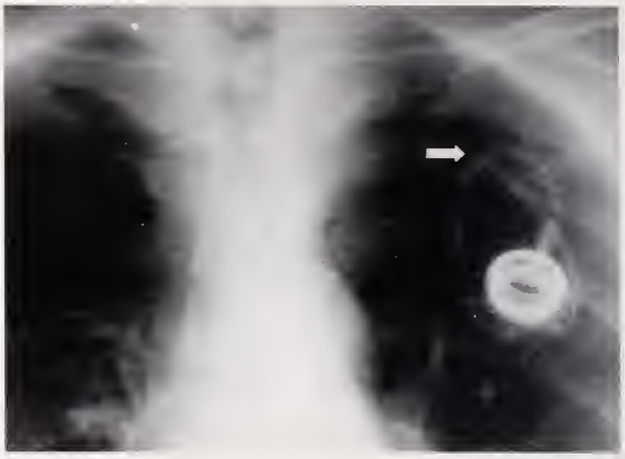

Fig. 2. This x-ray shows that the catheter (arrow) has migrated out of the vascular system and lies curled up in the subcutaneous pocket.

up, showed the catheter component of the SIP to be completely out of the venous system and coiled within the infraclavicular subcutaneous pocket (Fig. 2).

The SIP was subsequently removed and a new one was inserted using the same pocket. The catheter was repositioned into the superior vena cava via the left subclavian vein, as described above. This time, a silk suture was used to affix the catheter to the pectoralis fascia. The patient has subsequently done well.

Patient 2: C.S. is a 28-year-old obese woman who had a SIP placed because of poor venous access and frequent soft tissue infections requiring intravenous antibiotic treatment. No immunologic disorder had been identified on extensive work-up.

The patient's SIP was working well for about six weeks, at which time it suddenly could not be accessed. The diaphragm of the SIP was not palpable. Attempts to access the port were met with metallic resistance. Chest radiographs performed in the PA and lateral projections identified that the diaphragm of the SIP had flipped and come to rest against the chest wall (Figs. 3 and 4A). The proper position of the SIP is shown in Fig. 4B.

The patient was brought to the operating room, where the subcutaneous pocket was opened and the SIP flipped back into the correct position. It was secured to the fascia with absorbable 2–0 Vicryl® (Ethicon, Somerville, NJ) sutures. The SIP functioned well until six months later, at which time the scenario described above repeated itself. Chest x-ray confirmed that the SIP had flipped again. On subsequent exploration, a tight capsule was noted around the port; the capsule was firmly fixed to the surrounding tissues. This time, the SIP was secured to the fascia with 2–0 silk sutures. To date, the patient has not had any further problems.

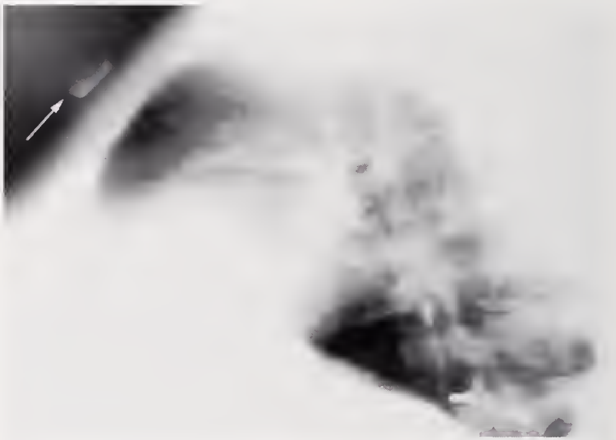

Fig. 3. The injection surface of this port (arrow) has flipped so that it now lies against the chest wall. When the needle is inserted, it can be felt to make contact with the metal posterior surface of the port.

Discussion

No prior cases of complete extravascular migration of a SIP catheter have been reported. The exact cause of the migration remains unclear. SIPs do not have a portion external to the skin such as that found in Hickman and Broviac catheters. Since the SIP is sutured to the pectoralis fascia, it is unlikely that enough external traction can be applied to cause the catheter to withdraw completely from the subclavian vein. Our patients gave no history of strenuous exercise, as had several patients who suffered partial migration or breakage of their SIP catheters (9).

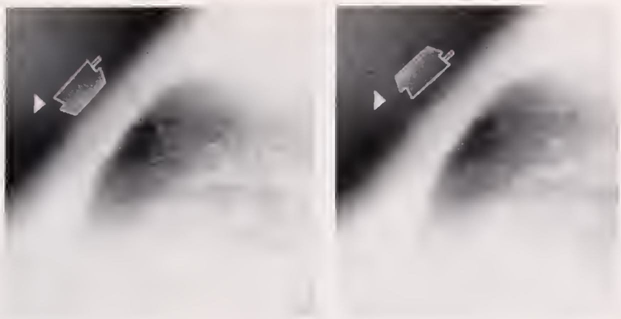

Fig. 4. **(A)** The port has been electronically outlined to indicate its flipped position, as shown in Fig. 3. **(B)** Proper position of the port is shown; the injection surface faces anteriorly and is directly under the skin.

Several points are worth mentioning. When an increased resistance to flow via the SIP is recognized, it is common practice to first flush the unit and catheter with urokinase; this is based on the assumption that any thrombus present within the unit and catheter will be dissolved. This report demonstrates the need to consider the possibility of catheter tip migration or port inversion when a SIP fails to function properly. Extravascular infusion of a caustic medication, such as Adriamycin, could have disastrous effects. Therefore, verification of the catheter tip and port position by physical examination and/or diagnostic study (i.e., AP and lateral chest radiograph) should be accomplished before infusing urokinase or any other medication.

In our own practice, absorbable sutures were always used to anchor the port to the pectoralis fascia, in the belief that the normal capsule that forms around the port would hold it in place subsequent to suture absorption. Since these events, we have modified our technique by using nonabsorbable sutures to anchor the port. We now routinely anchor the catheter portion of a SIP to the pectoralis fascia with a silk suture, as close to the inferior border of the clavicle as possible, in an effort to prevent catheter migration. Care is taken to avoid constricting the catheter lumen. This should reduce the likelihood of extravascular catheter migration.

Conclusion

SIPs have been shown to be a reliable means of long-term central venous access for the patients who require it. As with any medical device, new complications will continue to occur with increasing patient-years of usage. Our experience has shown the potential for total extravascular migration of the catheter portion of a SIP after implantation via conventional methods. We have also seen the SIP flip so that access to the diaphragm side, which now faces the chest wall, is not possible. By anchoring the catheter and SIP in place with nonabsorbable sutures, the potential for extravascular migration and flipping should be minimized. Since SIPs are likely to remain a mainstay of treatment in the future for those who require long-term central venous access, greater knowledge of problems which are encountered, and their solutions, is important.

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Gynandroblastoma in Pregnancy:

Case Report and Review of Literature

Tamara Kalir, M.D., PH.D., [1, 2] and Frederick Friedman, Jr., M.D.[2]

Abstract

Gynandroblastoma is an extremely rare tumor, composed of sex cord and stromal cells of both ovarian (granulosa-theca) and testicular (Sertoli-Leydig) types. We believe that its occurrence during pregnancy has not been previously reported. The patient was a 32-year-old woman who during her pregnancy was noted to have a progressively enlarging, unilocular left ovarian cyst. Beginning at 18 weeks gestation, the fetus required multiple platelet transfusions for severe alloimmune thrombocytopenia. A viable baby girl was delivered by cesarean section at 39 weeks gestation. At that time, an ovarian cystectomy also was performed. When the histology of the tissue subsequently became known, a left salpingo-oophorectomy was performed for gynandroblastoma. One year later, at the time of laparoscopic sterilization, the examination of the pelvis was normal. **Key Words:** Gynandroblastoma, ovary, pregnancy, case report.

Introduction

Gynandroblastoma is an extremely rare tumor. According to World Health Organization criteria, diagnosis should be made only if well-differentiated ovarian and testicular-type cells are present, each constituting a prominent component (at least 10%) of the neoplasm (1, 2). Of all cases reported, fewer than thirty have been accepted, upon review, as true gynandroblastomas (3, 4). Previously reported cases of gynandroblastoma are summarized in the Table.

Patients have ranged in age from 14 to 65 years. Presentations vary from being asymptomatic (incidental discovery) to complaints of amenorrhea, oligomenorrhea, menometrorrhagia, or postmenopausal bleeding. Some patients had evidence of hyperestrinism (endometrial hyperplasia) and others, of virilization. In patients of reproductive age, some have been nulliparous and others have had children. Nulliparous women complained of menstrual irregularities and/or hirsutism. Parous women reported secondary amenorrhea or menometrorrhagia. We believe that this is the first report of a gynandroblastoma discovered in a pregnant woman at the time of cesarean section.

Case Report

The patient was a 32-year-old woman who, three years previously, delivered at term a healthy child by cesarean section because of cephalopelvic disproportion. The patient had conceived without difficulty. That pregnancy had been complicated by mild fetal alloimmune thrombocytopenia (platelet equivalent of Rh sensitization) followed by percutaneous umbilical blood sampling (PUBS). The patient conceived again without difficulty. A first trimester ultrasound, although unremarkable, showed a 2 cm unilocular left ovarian cyst which was thought to represent a corpus luteum. At 18 weeks gestation, treatment for fetal alloimmune thrombocytopenia was necessary; it included fetal PUBS, platelet transfusions, and the administration of corticosteroids and gamma globulin to the mother.

From the Departments of [1]Pathology, and [2]Obstetrics, Gynecology, and Reproductive Science, Mount Sinai School of Medicine, New York, NY. Address correspondence to Dr. Tamara Kalir, Box 1194, Mount Sinai School of Medicine, One East 100th Street, New York, NY 10029.

TABLE

SUMMARY OF PREVIOUS REPORTS OF GYNANDROBLASTOMA

Reference	Patient Age (yrs)	Symptoms	Obstetrical History	Outcome	Tumor size cm	Tumor appearance
2	17	amenorrhea, viralization	G0	NED	6	solid
3	24	amenorrhea, hirsutism	G0	NED 2.5 yrs	8.5	cystic & solid
	30	oligomenorrhea, acne	N.E.	NED	5.5	multicystic
5	18	virilization	G0	NED 1 yr	>50	cyst
6	59	PMB, hirsutism	G7P6	NED 3.5 mos	1	solid
7	36	sterility	G0	NED 1.5 yrs	3	solid
8	24	hirsutism, amenorrhea	G0	NED 16 mos	6	solid
9	17	abdominal swelling, polymenorrhea	G0	NED 19 mos	14	multicystic
10	24	virilization	G0	NED 17 mos	18	multicystic
11	14	amenorrhea, hirsutism	G0	NED 9 mos	2.5	solid
12	26	amenorrhea, hirsutism	G2P2	NED 26 mos	6	cystic & solid
	31	amenorrhea, hirsutism	G1P1	NED 1 yr	19	multicystic
	38	menometrorrhagia	G4P3	NED 8 mos	5	cystic & solid
	53	abdominal enlargement	N.S.	N.S.	20	cystic
	25	menometrorrhagia	G2P1	NED 15 yr	5.5	solid
13	18	amenorrhea, virilization	G0	died 6 mos.	"football"	N.S.
	47	amenorrhea, virilization	P2	NED	7	N.S.
	33	asymptomatic	P0	NED	7	N.S.
	23	amenorrhea	P2	NED	5	N.S.
	31	amenorrhea, virilization	P1	NED	19	N.S.
	55	PMB, endometrial hyperplasia	P0	died post-op	20	N.S.
	16	irregular menses (1/2 yr)	P0	NED	25	N.S.
	26	amenorrhea, endometrial hyperplasia	P5	NED	9	N.S.
14	65	PMB	G7P6	NED 8 mos	13	multicystic
15	59	PMB	N.S.	NED 2 yrs	14	cystic & solid

PMB = postmenopausal bleeding

N.S. not stated

NED no evidence of disease

GxPy = gravidity (number of pregnancies) and parity (number of children)

Progressive enlargement of the adnexal mass was noted. At 39 weeks gestation, an elective cesarean section was performed, at which time a viable 3620 gram baby girl with Apgar scores of 9 and 9 was delivered. A left ovarian cystectomy was also performed. Operative findings demonstrated a thin-walled, 15 cm cyst with no adhesions and no additional abnormalities. The ovarian cyst was unilocular and contained clear serous fluid. The wall measured 0.1 to 0.4 cm in thickness. Histologic sections of the cyst showed a multilayered lining of luteinized theca cells with pale cytoplasm devoid of pigment granules (Fig. 1). Within and throughout the cyst wall, areas of granulosa cells were admixed with areas of Sertoli-Leydig cells in approximately equal amounts, and were seen in all slide sections made (i.e., comprised at least 10% of the tumor). The areas of granulosa cells revealed them to be distributed in several patterns, including diffuse, macrofollicular, and microfollicular with Call-Exner bodies (Fig. 2). Intranuclear "grooving" was notable. The Sertoli-Leydig cell tumor areas showed several patterns, all well differentiated, including simple tubular, folliculome lipidique (Sertoli cell tumor with lipid storage), and complex tubular (Fig. 3). The tubules were surrounded by islands of eosinophilic Leydig-like cells (Fig. 4). Reinke crystalloids were not noted; however, numerous intracytoplasmic pigment granules were present.

Three months later, in view of the final pathologic findings, a left salpingo-oophorectomy and peritoneal washings were performed. The ovary, measuring 2.5 cm × 1.5 cm × 1.5 cm, was grossly unremarkable. Histologic examination showed several microscopic foci of residual tumor in the hilus, adjacent to the previous cystectomy site. The foci showed granulosa cells and Sertoli cells.

One year later, the patient underwent laparoscopic sterilization, at which time the pelvis appeared normal.

Discussion

Gynandroblastoma is an extremely rare tumor. Diagnosis requires the presence of histologically identifiable granulosa-theca and Sertoli-Leydig cell components, each comprising at least 10% of the tumor (1, 4). The presence in the tumor of both male and female sex cord/stromal elements (Sertoli and Leydig, and granulosa and theca cells, respectively), has led to much speculation on the origin of gynandroblastomas. Various hypotheses have been proposed, the most accepted of which favors derivation from indifferent gonadal mesenchymal cells (15), which might be thought of as having dual capabilities, in the form of sex-cord/stromal cell differentiation (11). The origin of these tumors might also in part be explained by the current thinking regarding sex differentiation—that male gonadal development is not due solely to Y chromosomal elements, as was once conjectured, but probably also requires

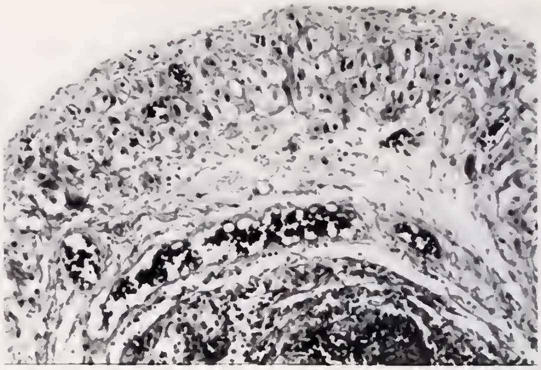

Fig. 1. Cyst lining showing luteinized theca cells, and associated blood vessels. 100X.

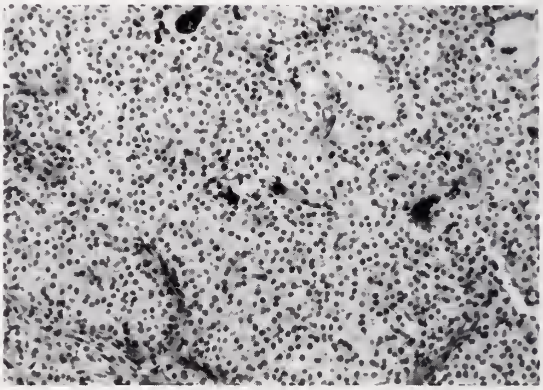

Fig. 2. Section from an area showing granulosa cell tumor, with several Call-Exner bodies. 100X.

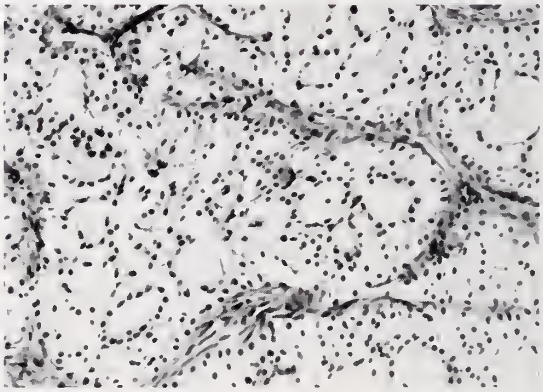

Fig. 3. Sertoli cell tubules, so-called complex pattern, showing cysts with discernible lumina. 100X.

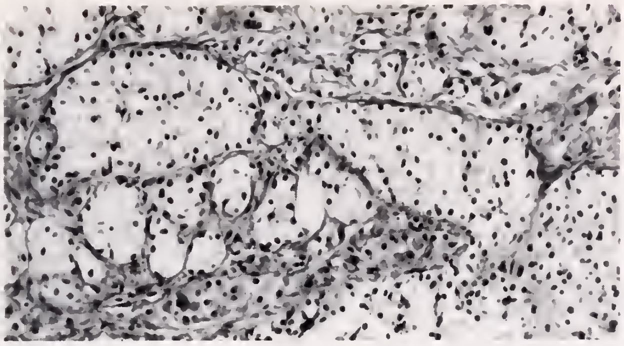

Fig. 4. Section from an area of the tumor showing solid Sertoli cell tubules (cells with clear cytoplasm) and adjacent strip of Leydig-like cells (cells with eosinophilic cytoplasm). 100X.

genes located on the X chromosome, and on autosomes (16–19).

In terms of hormonal activity of gynandroblastomas, virilization is expressed as hirsutism. In our table, 44% of the reported patients had symptoms that might be attributed to androgenic stimulation. A smaller proportion of the patients with tumors (30%) appeared with symptoms such as polymenorrhea or endometrial hyperplasia, suggesting an increase in estrogen production. Some patients (15%) reportedly experienced amenorrhea or oligomenorrhea, which may be related to either abnormal estrogen or abnormal androgen level. In some patients (4%), only abdominal enlargement was noted. In a few patients, including the current individual, the tumors have been asymptomatic (7%). Some authors have reported that there does not seem to be a relation between histologic appearance of the tumor and endocrinologic symptoms (12).

Most patients with gynandroblastoma have done well following resection of the involved adnexa. There have been no reports of metastasis (14, 15). However, in the series reported by Novak, one patient died of postoperative complications and another died with tumor (13). Further details regarding the latter patient's death are not given. Zhang (15) considers gynandroblastomas to be benign or of low malignant potential, but does not discuss the evidence upon which he bases this belief. Since granulosa cell tumors have been known to recur long after the initial resection, and since they comprise a component of gynandroblastomas, some authors (1, 9) advise periodic follow-up examinations in patients who have had gynandroblastomas removed. Since Sertoli-Leydig cell tumors can exhibit benign or malignant behavior, an important, but as yet unanswered, question remains as to whether a gynandroblastoma behaves as a combination of these two tumors or as a unique entity.

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Impact of the Libby Zion Case on Graduate Medical Education in Internal Medicine

Jeffrey M. Brensilver, M.D.,[1] Lawrence Smith, M.D.,[2] and Christopher S. Lyttle, M.A.[3]

Abstract

Residency training in New York State was substantially altered by the Libby Zion case. Work-hour limitations and augmented supervisory requirements changed the patterns of training—particularly in internal medicine—but with uncertain impacts on the quality of education and patient care. In this historical analysis, we review another major effect of the case: a substantial augmentation of the number of trainees. The need to maintain adequate inpatient staffing—within the ground rules of the Residency Review Committee, and in consideration of the reimbursement formulae and financial climate of New York State—conspired to promote substantial residency program expansion. Similar forces contributed to a national trend to increase the number of trainees. The history, cost and impact of these personnel changes are reviewed. **Key Words:** Graduate Medical Education (GME), training, manpower.

Introduction

Individual cases seldom catalyze dramatic nationwide changes in clinical medicine. Recent rare examples include the persistent vegetative state of Karen Quinlan and the occupational infection of a Johns Hopkins resident with HIV. At least as important as these is the now-famous case of one unfortunate young woman, Libby Zion, treated at a major New York City teaching hospital in 1984. In this paper we analyze the policy changes resulting from this case, and their impact on the costs, process and infrastructure of internal medicine training in the United States.

Case

On March 4, 1984, Libby Zion presented to a major New York hospital with fever and agitation. Less than 24 hours later, she died of an undiagnosed illness (1).

Although she was admitted to the service of a private physician, all care provided to her during her brief hospitalization was performed by the intern and resident on call that evening. The hospital course was one of increasing fever and agitation, culminating in cardiorespiratory arrest and death. Allegations of wrongdoing revolved around the use of meperidine in a patient who was taking an MAO-inhibitor, delays in the patient being seen by the house staff, the use of restraints, the lack of supervision, and the failure to make a diagnosis. The nature of the patient's illness and the cause of death have never been determined. Neither postmortem exam nor analysis of the data by many experts has ever elucidated exactly what happened to this unfortunate patient. After his daughter's death, Sidney Zion, an attorney, former federal prosecutor and newspaperman, conducted a campaign to correct the medical education system he believed culpable.

In 1987, a Manhattan grand jury was convened to evaluate the Libby Zion case and rule on the possibility of criminal wrongdoing. The grand jury

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[1] Director of Medicine, Sound Shore Medical Center of Westchester, New Rochelle, NY, [2] Program Director, Internal Medicine, Mount Sinai School of Medicine, New York, NY, [3] Center for Health Services Research, Rush Primary Care Institute, 1653 West Congress Parkway, Chicago, IL. Address correspondence to Dr. J. M. Brensilver, Sound Shore Medical Center of Westchester, 16 Guion Place, New Rochelle, NY 10802.

returned no criminal indictments. However, it sharply criticized the level of supervision of the house staff during the care of this patient. The grand jury also declared that there was a need for major reforms in the way house staff were trained. This led to the formation of the "Bell Commission," to study the problem (see next section).

Following the grand jury hearing, the Office of Professional Medical Conduct of the Department of Health of New York State initiated an investigation. After months of testimony, the panel concluded that the case was extremely complex, the postmortem findings inconclusive; expert witnesses were unable to provide definitive insight into the etiology of the illness. The panel recommended only censure of the involved physicians. This was approved by the New York State Commissioner of Health.

The next step in the medical misconduct proceedings was a review by the New York State Board of Regents. A subcommittee of the Board of Regents reviewed the testimony, reversed the Commissioner's ruling, and declared the intern and resident guilty of gross negligence of an egregious nature. The reversal of a Department of Health ruling, as well as the finding of gross negligence in a case involving physicians with no previous record of misconduct, represented an extraordinary break with precedent in New York State. The disciplinary action imposed by the Board of Regents, however, was restricted to that of censure and reprimand.

More recently, Sidney Zion pursued civil damages in the New York State Supreme Court. A jury concluded that there was shared negligence, equally divided between the physicians and the patient herself, for failing to disclose a full medication and drug usage history. A relatively small award was made. In addition, the jury found that the hospital and its methodology of training were not negligent (2). However, the major legacy of the Zion case, for better or worse, was the proposed changes to the resident training system.

The Bell Commission

The commissioner of health, the late Dr. David Axelrod, in response to the grand jury, convened a commission charged with developing rules that would prevent similar occurrences in the future. Axelrod, widely acknowledged as the architect of the nation's most comprehensive system of hospital regulation, appointed as Chair, Dr. Bertrand Bell, a physician-administrator, with a career interest in medical education.

The commission concluded that inadequate attending supervision, combined with impaired house staff judgment due to fatigue, were contributory causes of the patient's death. They recommended heightened requirements for attending supervision and improved ancillary support for residents. Further, they promulgated work-hour limitations for house staff and emergency room physicians. Trainees' total weekly work schedule would be limited to 80 hours. Consecutive hospital duty hours could not exceed 24 hours, and emergency room tours could not exceed 12 hours. Governor Mario Cuomo enacted such provisions in the state health code on October 1, 1988, effective July 1, 1989 (3, 4). In response to concerns about the incremental costs that would be incurred by hospitals (estimated at $358 million annually [5]), the legislature voted approximately $200 million per year to assist in complying with these regulations.

The New York State Response

The hospital/Graduate Medical Education (GME) community in New York, while applauding the goal of improved resident work conditions, responded vociferously to the proposed regulations (6, 7). Program directors raised concerns about the cost of enactment, the possible adverse effect on quality of care, the implications for education, and the expected need for an increased number of residents to provide adequate coverage with a reduced work-hour schedule.

Despite these objections, New York State's internal medicine community was forced to comply with the new law. Aggressive enforcement was threatened. This included the creation of a "hotline" for residents to anonymously report violations by their programs to the Department of Health. A number of clinical and economic factors set the stage for the obvious solution: expanding the size of programs. Important among these factors was a heavy reliance on house staff for the provision of care to the indigent. This resulted from the New York State formulae for Medicaid reimbursement, which fostered the concentration of Medicaid patients in teaching hospitals. Specifically, New York State provided extremely low Medicaid payment rates to private physicians providing service. Payments were more generous to hospital clinics, as were the GME payments to teaching hospitals (8). Thus, program directors could not easily adjust to reduced house staff availability by creating non-teaching services, as house staff were the principal direct care providers (under teaching supervision).

Also critical to the expansion of trainee numbers in medicine were the federal and state GME reimbursement formulae, which provided powerful incentives to increase the number of residents. The total reimbursement for each resident was very high in New York, reflecting historical regional cost adjustments from Medicare, and reflecting generous state payments to provide care to the indigent. In fact, GME reimbursement was, on average, close to $200,000 per resident in New York City area hospitals.

The national regulatory body for internal medicine training, the Residency Review Committee (RRC), had traditionally evaluated individual programs to ensure that educational resources were sufficient to provide adequate education to trainees. However, prior to 1994, the RRC in internal medicine did not set a specific maximum number of trainees for each program. Programs could expand or shrink as long as educational adequacy could be demonstrated at the time of program review (every 2–5 years). Thus, program expansion was virtually unregulated. The stage thus set, a dramatic increase in categorical (and preliminary) positions in internal medicine ensued (Fig. 1). However, general surgery program directors in New York, confronting the same dilemma, with one modifying constraint (an RRC limit on the numbers of residents), refused to comply. A letter of exception was crafted by the Department of Health to accommodate the special need for work-hour flexibility in surgery, basically exempting surgery programs from the impact of the new state regulations. Data supplied by the NYS Department of Health for the years 1988-1993 demonstrate that the number of internal medicine trainees increased by 24.6%; pediatrics, confronting similar forces, expanded by 29.6%. General surgery, in contrast, actually shrank by 2.5% (9).

Ironically, the $200 million earmarked by the state to mitigate the costs of adapting to work-hour regulations were irrelevant in view of the revenues guaranteed by program expansion. Although no data are available, anecdotal accounts suggest that most hospitals did not direct their portion of the $200 million to improving resident working conditions.

The National Response

The inherent wisdom of an 80-hour work week proved equally compelling at the national level. Thus, the RRC in internal medicine adopted work-hour limitations virtually identical to those in the New York State code. And program directors around the country identified the same solution as did their colleagues in New York. A dramatic nationwide increase in internal medicine positions (Fig. 2), may be ascribed largely to the need for more residents to provide the necessary coverage within the work-hour limitations. A review of data provided by the National Residency Matching Plan (NRMP) demonstrates steady, slow growth of internal medicine training since the late 70s, and between 1982 and 1987 the number of internal medicine residents had shown only modest growth. Truly substantial augmentation only occurred after the work-hour rules were changed. Many factors may have contributed to the nationwide augmentation after 1988, including the expansion of internal medicine experiences in ambulatory care and efforts to improve the quality of life of trainees. Certainly, the financial incentives provided by Medicare, combined with the absence of an RRC cap, encouraged the expansion of programs to ensure compliance with national work-hour limitations (10–13).

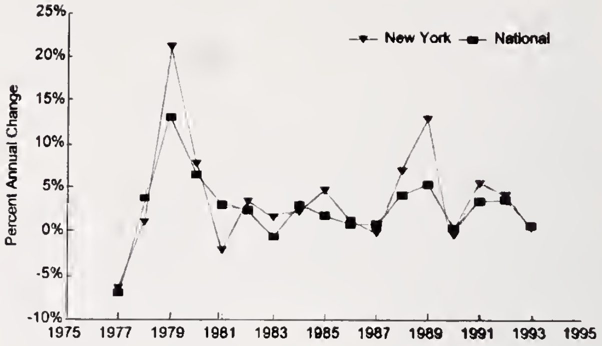

Fig. 1. Annual change in numbers of first-year internal medicine residents in New York State and in the United States.

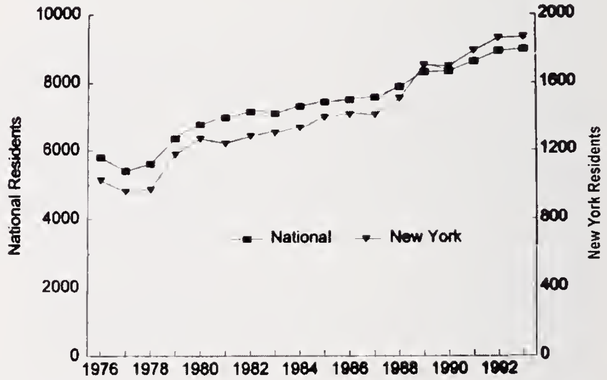

Fig. 2. Total numbers of first-year internal medicine residents in New York State and in the United States.

The State and National Impact

Cost: Between 1988 and 1993, approximately 500 first-year internal medicine positions were progressively added to the national pool (see Appendix). Most of these 500 residents would stay for 3 years. Thus, over a five-year period, approximately 3000 additional salaries were funded. Although the precise reimbursement level is difficult to ascertain, $80,000–$100,000 nationally per resident per year is a conservative estimate. Thus, as much as $300 million may have been added to educational costs resulting from the Bell Commission.

Training and Patient Care: The clinical and educational impact of work-hour restrictions in New York has been examined. Conigliaro et al. (14) surveyed internal medicine resident and attending attitudes two years after enactment of the 405 code changes. Although both groups of respondents agreed that resident quality of life had improved, they were both uncertain about the effects on quality of supervision and on resident intellectual achievement. Attendings expressed concern about the development of a shift-work mentality among house staff. Laine et al. (15), in a retrospective analysis at one major hospital, observed that patient care had not been substantially improved or damaged. However, they did identify an increased frequency of diagnostic test delays and inpatient complications. The NYS Department of Health had solicited bids for proposals to study the effects of the regulations, but funding was eliminated when a new governor took command of the state Department of Health. As no prospective study was designed at the time of the changes, it is doubtful that any firm data will ever be obtained.

Beyond the boundaries of New York, one study from Boston (16) has examined the relationship between adverse patient events and resident schedule. The authors reported a strong correlation between the frequency of adverse events and cross-coverage by residents of teams not directly responsible for the patient's care. They suggested that house staff cross-coverage schedules, necessitated by work-hour reform, must be carefully adjusted to avert patient complications.

Physician Supply: As residency programs, both in New York State and around the country, rapidly expanded, a marked imbalance arose between the number of residency positions offered and the number of United States medical graduates available and interested in filling these positions (17). Thus, there was a major influx of international medical graduates, many of whom remained in the United States following the conclusion of their residency training. This has contributed to a perceived oversupply of physicians. In addition, many of these international medical graduate physicians pursued subspecialty training, which added to a previous imbalance between specialists and generalists.

Ironically, reflecting new attitudes regarding health-care reform, the 1997 New York State Health Code has created incentives for *reducing* the number of residency positions in New York (18). In addition, a recently enacted demonstration project (sponsored by the Health Care Finance Administration) will foster a 20-25% overall reduction in trainees in the majority of hospitals in New York State (19). Reduction in house staff numbers, combined with continued enforcement of the New York State work-hours regulations, will put enormous pressure on all New York State training programs to maintain educational integrity, patient care, and work-hour restrictions.

Conclusion

We submit that the Bell Commission's response to a remarkably complex individual clinical scenario set in motion a series of actions which contributed to a substantial nationwide augmentation of the size of internal medicine training programs. Specifically, work-hour restrictions spawned a creative local solution—passing the cost to Medicare by program expansion—as allowed by the absence of a specific RRC cap on residency positions. The national medicine community of residency training programs in internal medicine accepted the wisdom of the Bell Commission's recommendations, and the RRC canonized this acceptance into policy. This chain of events, initiated by appointed state health officials (in a state with a strong commitment to hospital regulation), could not have been easily foreseen. While the need to downsize the number of trainees has been a dominant theme of medical education in the mid-90s, the origin of the increase in positions has not been squarely acknowledged. The sequence of events triggered by the Bell Commission dramatically increased the cost of internal medicine graduate medical education, radically increased reliance on international medical graduates, and added to potential doctor oversupply. Whether education has been improved by work-hour rules is open to speculation. Whether any patients have been saved by a better-rested resident staff will never be known. Clearly, however, residency training in internal medicine has been radically changed by a tragic event experienced by a single patient.

Appendix

The calculation of additional first-year internal medicine residents trained nationally proceeded by establishing a base rate of increase, using the years 1981–1982 to 1987–1988. This base rate (1.62%) was used to calculate projected first-year resident populations for 1988–1989 to 1993–1994, building on the observed 1987-1988 data. The excess of observed over projected first-year residents for these years (192 in 1988–1989, 490 in 1989–1990, 395 in 1990–1991, 555 in 1991–1992, 736 in 1992-1993, and 664 in 1993–1994) totals slightly more than 3000.

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The Libby Zion Case Revisited:

What Have We Learned?

Barry Stimmel, M.D.

That the unfortunate case of Libby Zion, a young woman who died within twenty-four hours of being admitted to a major medical center in New York City, was responsible for major changes in residency training in New York State is unarguable. These changes may have had a "ripple effect" on the nationwide training of residents. At least equally important, this case also broke legal precedents: a grand jury convened to evaluate the possibility of criminal wrongdoing by the house staff and the attending physician who had cared for Ms. Zion; and the New York State Board of Regents overturned rulings of the New York State Office of Professional Medical Conduct and the New York State Commissioner of Health, to declare that those involved with Ms. Zion's care were guilty of gross negligence. As a result of this case, regulations concerning the supervision of house staff were severely tightened, and the work hours of house staff were curtailed to a maximum of 80 hours per week, with no more than 24 hours on call at any time. This revision of Part 405 of the State Health Code (1) had as its main goals improvement in the quality of life and working conditions of residents, in the supervision by attending physicians, and in the delivery of patient care.

It is impossible to argue against either better supervision by attending physicians or more humane working conditions for house staff. However, it remains unclear whether these regulations were indirectly responsible for the current physician surplus, as suggested by Brensilver and colleagues in this issue of the *Journal* (2), or—more important—whether they have resulted in an improvement in the quality of care provided by house staff. On the surface it would appear that to satisfactorily address the changes in Section 405, programs had to increase the number of house staff. However, there were other alternatives. For example, program directors could choose to provide care to patients through means other than the use of house staff. A survey of more than 400 internal medicine residency programs found that only 27% had hired more residents. Other solutions included: (a) decreasing the number of electives (42%), (b) creating non-teaching services (12%), (c) reallocating resident time (10%), or perhaps (d) refusing to comply (3). The surgical specialties chose this last option—to conduct business as usual—and, in fact, received permission from the New York State Department of Health to do so. This waiver, although perhaps logistically understandable, is less comprehensible if it is really assumed that Ms. Zion's death was due to resident fatigue, as there are few residents who work as hard, with less sleep, than surgeons.

New York State provided millions of dollars for institutions to obtain alternate means of caring for patients. However, these funds paled by comparison with the amount of money that institutions could receive from federal reimbursement for resident education by merely increasing the numbers of house staff. In the medical specialties, at that time, this could be done quite easily, as contrasted with the surgical specialties, where specific approval by the appropriate resident review committee was needed. However, that the increase in medical residents was not solely related to the Libby Zion case can be seen from the figures presented by Brensilver et al., which

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Dean for Graduate Medical Education, Mount Sinai School of Medicine, New York, NY. Address correspondence to Barry Stimmel, M.D., Mount Sinai School of Medicine, Box 1193, One East 100th Street, New York, NY 10029.

demonstrate a marked annual increase in first-year internal medicine residents in 1979, with subsequent smaller increases in 1985 and 1991. In fact, the total number of first-year internal medicine residents, both in New York State and nationally, has progressively increased since 1978, only recently leveling off in 1996. These increases, independent of the Libby Zion case, are more closely related to the need to provide increased time in ambulatory care training, and to the system of GME reimbursement, which on an annual basis can exceed $225,000 per resident per year. In fact, it was only in the 1997 fiscal year, with passage of the Budget Reconciliation Bill, that an absolute institutional cap on the number of house staff trained was established.

Although there is no question that work restrictions have improved the quality of residency life (4), and that the need for "on-site" attending physicians has had a beneficial effect on resident supervision, it remains to be determined whether the quality of care provided to patients has comparably improved. Those few studies performed could not find such relationships, with some actually identifying an increased frequency of diagnostic test and inpatient complications (5–7). Anecdotal stories from physicians suggest that the alternate method of providing house staff coverage through night floats or day floats has disrupted, rather than enhanced, the continuity of care. Although the state Health Department had planned to evaluate these changes, for reasons less than clear this decision was reversed, and as noted by Brensilver et al., it is unlikely that any firm data will be obtained.

In summary then, it is unclear whether the events triggered by the Libby Zion case resulted in an increase in house staff in the medical specialties in New York State, or whether other factors, which had their impact on a national level, were responsible for what is now believed by virtually all professional groups to be an increasing oversupply of physicians. With respect to institutions within New York State, it is quite encouraging that, due to the efforts of the federal Health Care Finance Administration, the New York State Reform Health Care Act, and the commitment of many institutions to "rightsize" the house-staff work force, these issues are being addressed. Other institutions in other states have also initiated this process, and on a national level through the Balanced Budget Act of 1997 (8), Congress has finally placed a "cap" on the numbers of residents being trained as well as provided a national incentive for downsizing.

Finally, were the working conditions addressed by Section 405 really related to the death of Ms. Zion, or was the inappropriate use of restraints, the failure to respond to calls for assessment, or the lack of knowledge of interactions between monoamine oxidase inhibitors and meperidine more directly responsible? Here again, we will never know the answer(s) with certainty. But remembering this case and discussing it with each group of entering house staff is essential. The importance of constant vigilance and concern when a person fails to respond to the initial care provided, as well as the limitations and hazards of the casual use of restraints, must never be forgotten.

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Authors' Reply:

As Dr. Stimmel emphasizes, the Bell regulations, developed in response to a high profile tragedy, forced teaching hospitals to confront deficiencies in resident supervision and to address the exploitative excesses of resident work schedules. Our historical analysis is not intended to identify villains or heroes. The protagonists of this drama, hospital administrators, training program directors, Libby Zion's father, the State regulatory agencies, and the RRC all reacted in logical, predictable fashion to the economic, professional, and emotional forces unleashed by the events leading to Ms. Zion's death.

We hope to provoke in the medical education community a reexamination of these events in the case-based style of internal medicine. Dr. Stimmel has suggested that programs teach the Libby Zion case to residents, so that trainees may learn cautionary tales about the use of restraints, drug interactions, and the need to seek help with complex diagnostic illnesses. We suggest that medical educators also review the secondary, nationwide implications of the case. Although there were many factors facilitating the increase in house staff number (including the generous medicine IME and GME funding), the temporal relationship between the increase in size of programs in New York State and the imposition of the 405 Regulations seems more than coincidental.

Most program directors would agree that erratic supervision of residents and overreliance on trainees for service were troublesome, festering problems which were well known in 1987. We depended on bright, highly motivated residents to use their creative problem-solving abilities to overcome systems deficiencies. Lucien Leape's eloquent analogy from the airline industry is relevant (1). When novice pilots are provided with irregularly maintained aircraft and instructors are available only by telephone, we should not be surprised by periodic crashes. The challenge for medical educators is to learn from the Zion case to proactively improve local systems, to address the problems created by downsizing, reduced funding and managed care.

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Student Corner

Unexpected Factors Predict Control of Hypertension in a Hospital-Based Homeless Clinic

JOSIAH CHILD, M.D.,[1] MICHAEL BIERER, M.D., M.P.H.,[2] AND KIM EAGLE, M.D.[3]

Abstract

Background: Boston Health Care for the Homeless Program (BHCHP) physicians conduct a primary care clinic twice a week at Massachusetts General Hospital (MGH). The MGH clinic is part of a city-wide network of BHCHP clinics providing primary care services to indigent patients. Despite this network, long term control of chronic illnesses such as hypertension (HTN) continues to challenge the clinic staff.

Methods: In an effort to better understand the factors obstructing long term treatment of chronic illnesses, we conducted a chart review of hypertensive patients seen over a three-year period (January 1991 to March 1994) at the MGH clinic. Frequency of visits, total number of visits and physicians' notes on concomitant diagnoses were analyzed for their correlation to control of hypertension.

Results: Overall control of hypertension was poor (42%). A greater proportion of patients with a diagnosis of psychiatric illness responded to treatment intended to lower their blood pressure below 140/90 mm Hg than those without such a diagnosis (odds ratio: 10.2). While there was no difference in the total number of clinic visits during the study period, those with a diagnosis of psychiatric illness had a lower average number of days between their first and third visits (52 days vs 108 $p=0.002$).

Conclusions: A greater proportion of patients with concomitant psychiatric diagnoses exhibited blood pressures ≤ 140/90 mm Hg than patients without mental illness. The increased frequency of visits at the onset of treatment may confer a positive effect on long term control of HTN among homeless patients attending outpatient hospital-based clinics. **Key Words**: Hypertension, control, compliance, homeless persons, psychiatric.

Introduction

MEETING THE SPECIAL NEEDS of homeless patients has been a challenge for health care institutions and has led to the creation of special programs. Federal support for the 123 designated homeless health care programs nationwide has risen to $63 million in 1994, representing a 4-fold increase in the annual appropriation over that appropriated 5 years earlier (1). Despite the substantial and growing resources devoted to the care of homeless people, little is known about their chronic medical conditions or how they are managed.

Hypertension (HTN) is the most common chronic illness among people who are homeless (2). Yet, because it is an asymptomatic condition, motivating patients to adhere to long-term regimens is often a difficult task for health care providers. It is therefore useful to identify means by which both outpatient clinics and individual health care practitioners can improve the long-term management of hypertension (HTN). Such information may be used not only to improve long-term management of hypertension itself but also the treatment of other asymptomatic illnesses

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[1]Mount Sinai School of Medicine, New York, NY, [2]Boston Health Care for the Homeless Program, Massachusetts General Hospital, General Internal Medicine Unit, Boston, MA, and [3]Michigan Heart Care Program, University of Michigan, Ann Arbor, MI. Address correspondence to Josiah Child, M.D., 230 Everet Street, New Haven, CT 06511.

common among this population such as tuberculosis and early HIV infection.

Methods

A focus group of Boston Health Care for the Homeless (BHCH) practitioners met to predict factors that they believed affected long term control of blood pressure. Categories included: age, race, weight, comorbidities, psychiatric illness, substance abuse, reasons for visits, frequency of visits, sleeping places (shelters or a friend's apartment versus the street). Practitioners also expressed the overwhelming opinion that regimens in which medication(s) were taken once daily ensured better compliance than twice daily regimens. However, the practice of taking medications once daily was so widespread its efficacy could not be tested.

Subsequently, clinic charts of 252 patients who presented for clinic visits between January 1, 1991 and March 28, 1994 were reviewed. Patients were defined as hypertensive if they: a) presented in clinic with a blood pressure greater than 140/90 mm Hg on two consecutive occasions (in most cases this occurred one week later); b) were taking antihypertensive medications or c) carried a diagnosis of HTN in their chart. The endpoint measured was control of HTN, defined as a blood pressure < 140/90 mm Hg during their last two visits. Any ambiguities in a patient's chart were resolved by contacting the patient's treating physician.

Statistical Analysis

The correlation between a patient's status in each of the hypothesized factors mentioned above (substance abuse Y/N, psychiatric illness Y/N, etc.) and control of blood pressure was analyzed by setting up multiple two-by-two tables (Table). P values were determined using the Fischer's Exact Test. Reliability of the chart review data was analyzed by comparing the results of two reviewers' analysis of each chart. The presence or absence of the diagnosis of psychiatric illness was chosen to assess interobserver variability. It was determined that the reviewers were in substantial agreement (Kappa Score=66) concerning the identification of psychiatric illness (3).

Results

Treatment

No dietary or lifestyle modification strategies (such as exercise) were noted for the treatment of HTN; no trends in weight gain or loss were noted in any of the subjects. Twenty patients (41%) were prescribed calcium channel blockers, 14 (29%) were prescribed β-blockers, 8 (17%) were on combined regimens, 4 (10%) were prescribed diuretics alone and 2 (4%) were prescribed α-blockers. There were no differences in outcomes noted between these treatment groups.

TABLE

Response of Blood Pressure to Treatment

	Total Patients n=45	Uncontrolled n=26	Controlled n=19
No Diagnosis of Psychiatric Illness	23	19	4 *
Diagnosed Psychiatric Illness	22	7	15 *
White	23	12	11
Black	11	7	4
Hispanic	8	6	2
Asian	1		1
Unknown	2	2	
Alcohol or drug problem	23	15	8
No record of substance abuse	22	10	12

* p=0.002

Nine hypertensive cases received no antihypertensive medications either because the patient was not in compliance (n=7) or the doctor did not think it appropriate to initiate therapy because of complicating factors (n=2). Of the 7 cases of reported noncompliance, 2 (29%) were eventually successful in bringing down their blood pressure below 140/90 mm Hg. In 2 cases, patients met our definition of hypertension but the patient's physician did not initiate treatment. In both cases the patient had significant personal, medical and psychiatric problems that resulted in the physician not recommending treatment. These patients were not excluded from analysis.

Three patients visited the clinic only once during the 3-year period. These 3 patients lived in other states and visited the clinic, presumably, only to renew prescriptions. They were not included in the analysis.

Blood Pressure Control

Overall, in 19 of 45 patients (42%), the blood pressure declined to less than 140/90 mm Hg. Twenty-two of these patients (49%) carried a diagnosis of psychiatric illness. Of this latter group of 22 patients, blood pressure decreased to less than 140/90 mm Hg in 15 (68%). In contrast, in only 4 of the 23 patients (17%) without a diagnosis of psychiatric illness did the blood pressure decrease to below 140/90 mm Hg (Table, Odds ratio: 10.2, p=0.002).

The average interval between visits for the patients with psychiatric illness was 52 days compared to 119 for those patients without psychiatric illness ($p \leq 0.002$). There was no difference in the total number of clinic visits in those whose blood pressure declined versus those in whom the blood pressure did not change. Also, there was no difference in the two groups with respect to the average length of follow-up.

Demographics

The proportion of patients in whom blood pressure decreased below 140/90 mm Hg was lower among Hispanics 2/8 (25%) and Blacks 4/11 (36%) than among Whites 11/23 (48%) (Table). Of the patients noted to have abused alcohol, the blood pressure fell to below 140/90 mm Hg in 8/23 (35%) in contrast to 10/21 (48%) of those without alcohol abuse as noted in their chart. The association did not reach statistical significance.

Discussion

Control

Homeless patients live in a chaotic and often hostile environment that may interfere with the long term treatment of hypertension. Patients forget appointments (4), perceive the importance of disease prevention as small in comparison to other pressures, have difficulties obtaining a healthy diet (5), and finding safe places to store medications. Lack of structural or familial support to motivate patients to adhere to medical regimens may play a role in decreasing patient compliance and therefore the control of hypertension (6). The overall rate of response to treatment of 42% observed in this study is consonant with other studies (7, 8) as compared to 60% among domiciled patients when other factors have been controlled for (9).

It is unclear why the blood pressure was lowered in a greater proportion of patients with psychiatric illness. Those patients with a diagnosis of psychiatric illness tended to see their primary care provider more frequently initially even though the final number of visits was similar to non-psychiatric patients. It is possible that by scheduling frequent visits at the onset of treatment, lasting habits were formed. It is also possible, however, that those patients carrying a psychiatric diagnosis were involved in day treatment programs that emphasized following medical regimens. Alternatively, clinicians may have emphasized the need for compliance with medical regimens in those patients with a psychiatric diagnosis.

It is also possible that those patients with a diagnosis of psychiatric illnesses tended to be the beneficiaries of informal counseling sessions with their primary care providers, which may have improved their compliance to medication regimens or had a more direct role in lowering their blood pressure. It has been suggested that the use of mental health workers or other counselors as an adjunct to physician services may be effective in improving control of long term illness in clinics (10). Interestingly, one study showed that interventions staffed by trained lay workers have proven to be as effective in improving compliance as those staffed with professionals (1). Syme (12) demonstrated that simply talking with a patient once a week about any subject had a positive effect on the control of hypertension even after the influence of improved compliance was discounted. When viewed in the context of these previous studies our results support the highly plausible hypothesis that the doctor-patient relationship may play an especially important role in the effective treatment of homeless patients with chronic asymptomatic illnesses.

Conclusions

Several observations from this study deserve emphasis. A greater proportion of hypertensive patients with concomitant psychiatric illness responded to hypertensive treatment regimens with a decline in blood pressure than patients without mental illness. These patients also visited the clinic more frequently at the onset of their treatment. Further studies are needed to determine whether increased frequency of clinic visits, counseling sessions or regimented daily distribution of medications are effective in improving the efficacy of the treatment of chronic illnesses among homeless patients.

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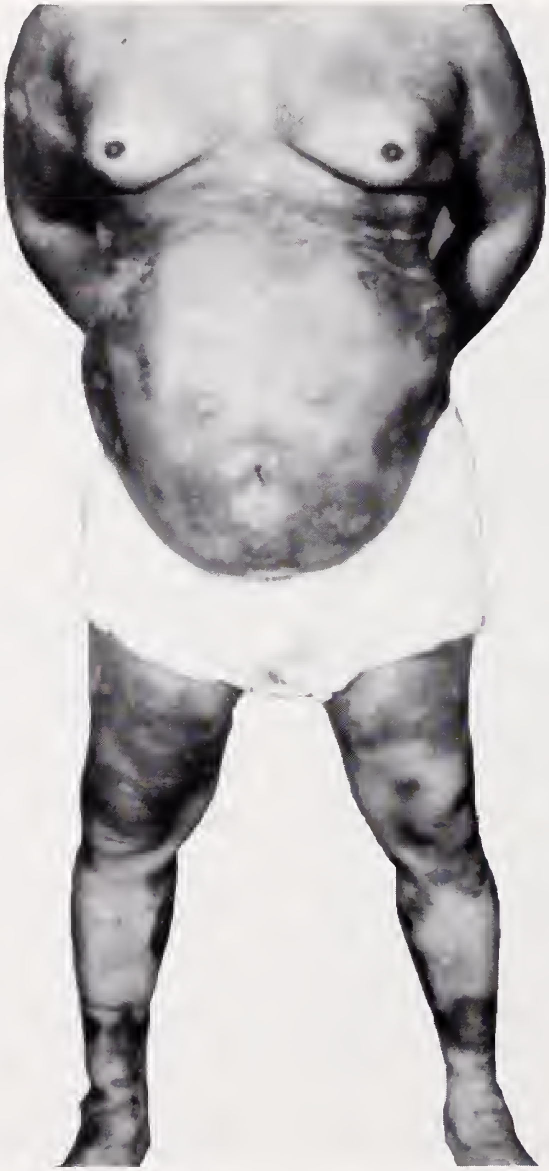
Generalized Morphea



GENERALIZED INDURATED, hypopigmented plaques are evident on the patient's breasts, abdomen, and legs. Generalized morphea can be distinguished from systemic scleroderma by several features. It is not associated with any of the systemic manifestations of progressive systemic sclerosis such as renal failure and hypertension. Patients usually do not develop sclerodactyly. Also, Raynaud's phenomenon, which uniformly occurs in systemic scleroderma, does not occur in patients with generalized morphea.

—Marsha Gordon, M.D., and Mark Lebwohl, M.D.

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CONTENTS

Editor's Note *Sherman Kupfer* i

VOLUME 65 NUMBER 5 OCTOBER/NOVEMBER 1998

THEME ISSUE
ORAL MEDICINE *Vincent B. Ziccardi, guest editor*

Oral Manifestations of Systemic Diseases
Richard G. Long, Lubor Hlousek, and John L. Doyle 309

Implant Reconstruction of the Jaws and Craniofacial Skeleton
Pamela L. Alberto 316

Oral Manifestations of HIV Disease *David A. Sirois* 322

Minor Oral Injuries in Children *Kathy Banks and Phyllis G. Merlino* 333

Burning Mouth Syndrome *Steven A. Miyamoto and Vincent B. Ziccardi* 343

Differential Diagnosis of Orofacial Pain *Richard A. Pertes* 348

Oral Manifestations of Nutrient Deficiencies *Riva Touger-Decker* 355

Periodontal Disease: An Overview for Physicians *Kim E. Fenesy* 362

Oral Cancer and Oral Effects of Anticancer Therapy
Andrei Barasch, Monika Safford, and Ellen Eisenberg 370

Transmission of Infectious Disease in the Dental Setting
Jagdev Heir and Vincent B. Ziccardi 378

Diagnosis of Oral Ulcers
Lawrence C. Schneider and Adina E. Schneider 383

Antibiotic Prophylaxis in Dentistry *André Montazem* 388

VOLUME 65 NUMBER 6

GENERAL ARTICLES

The Impact of Language as a Barrier to Effective Health Care in an Underserved Urban Hispanic Community
Rand A. David and Michelle Rhee 393

The Importance of the Family Context in Inflammatory Bowel Disease
Mary-Joan Gerson, Jacqueline Schonholtz, Catherine H. Grega, and Deborah R. Barr 398

Two-Level Thoracic Disc Herniation *Niels Levi and Kjeld Dons* 404

Judaism, Genetic Screening and Genetic Therapy *Fred Rosner* 406

CLINICOPATHOLOGICAL CONFERENCE The Mount Sinai Hospital
Michael E. Blam, Editor; Noel Mann, Michael Kim, and Dushyant P. Purohit 414

Value of Assessing Cryptococcal Antigen in Bronchoalveolar Lavage and Sputum Specimens from Patients with AIDS *Edward J. Bottone, Monica Sindone, and Victor Caraballo* 422

DERMATOLOGY NOTES
Pyoderma Gangrenosum *Michael Fellner and Mark Lebwohl.* 426

List of Journal Reviewers in 1998 427

Author, Subject, Title Index to Volume 65 428

Information for Authors ii

Required Form for Authors C3

Editor's Note

With this issue, *The Journal* is reinstituting a feature which was included for many years and which, apparently, fell out of favor. Perhaps this may have been due, in part, to the marked decline in autopsy rates, as well as the growth and availability of sophisticated laboratory procedures including analyses of tissues obtained by biopsy via a percutaneous needle or endoscope. We expect that many of our interesting case presentations will be based on biopsy material and laboratory analyses. These developments and the evolution of reliable laboratory procedures have been a great boon to the quality of patient care, medical practice and medical education.

However, many cases remain in which these procedures have not always clarified the diagnoses and in which no post-mortem examinations had been performed. The anguish and emotional stress imposed on families, next of kin, and attending physicians associated with the procedure of obtaining consent for a post-mortem examination are understandably significant hurdles to be overcome. Surely, the post-mortem examination of tissues remains critical in evaluating the differential diagnoses and the effects of therapy. Any view that the post-mortem examination of tissues is not worthwhile, despite its expense, is just plain wrong. Without doubt, the results of any post-mortem examination must be incorporated into the assessment of the quality of care delivered to the specific patient, by personnel of the institution. Can we, as physicians, be sure that the history and physical examination provided the necessary clues? Or was an important detail overlooked? This exercise remains an important tool in our learning and in teaching our students. This feature will be directed towards these ends.

Sherman Kupfer, M.D.
Editor-in-Chief

Oral Manifestations of Systemic Diseases

Richard G. Long, D.D.S.[1], Lubor Hlousek, M.D., D.M.D.[1], and John L. Doyle, D.D.S., M.Sc.D.[2]

Abstract

Many systemic diseases have oral manifestations. The oral cavity might well be thought of as the window to the body because oral manifestations accompany many systemic diseases. These oral manifestations must be properly recognized if the patient is to receive appropriate diagnosis and referral for treatment. We have reviewed a series of recent articles and summarized known and newly described oral manifestations of several systemic diseases. The lesions of the oral mucosa, tongue, gingiva, dentition, periodontium, salivary glands, facial skeleton, extraoral skin and other related structures caused by some of the more common systemic diseases are highlighted. **Key Words:** Oral manifestations, anemia, leukemia, rheumatology, neoplasm, metastasis, diabetes mellitus, uremia, Crohn's disease, endocrinology.

Hematologic Diseases

Anemia

Iron deficiency anemia is the most common hematologic disorder (1). Oral manifestations include atrophic glossitis, mucosal pallor, and angular cheilitis. Atrophic glossitis, flattening of the tongue papillae, resulting in a smooth and erythematous tongue may mimic migratory glossitis. Migratory glossitis, also known as geographic tongue, is a condition of unknown etiology that affects 1–2% of the population. It results in lesions on the tongue that are erythematous, non-indurated, atrophic, and bordered by a slightly elevated, distinct rim that varies in color from gray to white. In atrophic glossitis, these areas do not have a white keratotic border and they increase in size rather than changing position. In more severe cases, the tongue may be tender. Angular cheilitis, occurring at the commissures of the lips, is caused by *Candida albicans* infection (2) resulting in reddening and cracking, and some level of discomfort. The manifestations of Plummer-Vinson syndrome also include dysphagia due to pharyngoesophageal ulcerations. Oral complications seen with sickle cell anemia include mandibular salmonella osteomyelitis, which results in areas of osteoporosis and erosion followed by osteosclerosis. Anesthesia or paresthesia of the mandibular nerve, and asymptomatic pulpal necrosis may also occur (3). These conditions are exacerbated when marrow proliferation is intense. Associated dentofacial deformities are characterized radiographically by areas of decreased densities and coarse trabecular pattern most easily seen between the root apices of the teeth and the inferior border of the mandible. Osteosclerosis may follow thrombosis and infarction.

Leukemia

Oral complications of leukemia frequently include gingival hypertrophy, petechiae, ecchymosis, mucosal ulcers, and hemorrhage (4). Less frequently, mental nerve neuropathy, called

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From the [1]Department of Oral and Maxillofacial Surgery and [2]Clinical Oral Pathology, Biology and Diagnostic Sciences, New Jersey Dental School, University of Medicine and Dentistry of New Jersey, Newark, NJ. [1]Resident and [2]Professor.

Address correspondence to John L. Doyle, D.D.S., M.Sc.D., University of Medicine and Dentistry of New Jersey, New Jersey Dental School, Department of Oral Pathology, Biology and Diagnostic Sciences, Room C-829, 110 Bergen Street, Newark, NJ 10703-2400.

"numb chin syndrome," may be the presenting complaint (5). Palatal ulcerations and necrosis may herald the presence of mucormycosis of the nasal cavity and the paranasal sinuses (6). Sixteen per cent and 8% of children with acute leukemia are reported to have gingivitis and mucositis respectively (7). Bacterial infections of the oral cavity, which may be a source of septicemia, are also common in these patients and must be recognized early and treated aggressively. Treatment of leukemia with chemotherapeutic agents can result in reactivation of herpes simplex virus (HSV) leading to oral mucositis. Oral mucositis can also occur from chemotherapy without an HSV component, since thinning of the surface layer of mucosa and/or bone marrow suppression allows for opportunistic organisms to invade the mucosa (Fig. 1).

Multiple Myeloma

When multiple myeloma involves the oral cavity, it is usually a late secondary manifestation of lesions within the jaws, most often the mandible. These lesions cause swelling of the jaws, pain, numbness, mobility of teeth, and pathologic fractures (8). Punched out lesions of the skull and jaw are characteristic radiographic findings. The incidence of jaw involvement in multiple myeloma averages about 15% (9–13). Since multiple myeloma results in immunosuppression, a variety of infections may be present, including oral hairy leukoplakia and candidiasis (14, 15). Amyloid deposits in the tongue can lead to macroglossia (16).

Rheumatological Diseases

Sjögren's Syndrome

Sjögren's syndrome (SS) patients frequently present with xerostomia and parotid gland enlargement (17). SS is often associated with rheumatoid arthritis. In one study (18), 88% of the patients with SS had abnormal submandibular/sublingual salivary flow, and 55% had abnormal parotid flow. Enlargement of the parotid or submandibular gland is apparent in 35% of patients with SS (19). Xerostomia can be associated with fissured tongue, depapillation and redness of the tongue, cheilitis, and candidiasis (Fig. 2). Swallowing and speaking may become difficult due to persistent xerostomia. Bacterial parotitis, which is usually accompanied by fever and purulent discharge from the gland, may also occur. There is an increase in dental caries, especially around the cervical region of the teeth (20). It is important to recognize SS quickly and refer the patient to a dentist since dental caries can progress rapidly (21). Diagnosis is usually confirmed by labial minor salivary gland biopsy. Histologically, there is a periductal lymphocytic infiltrate.

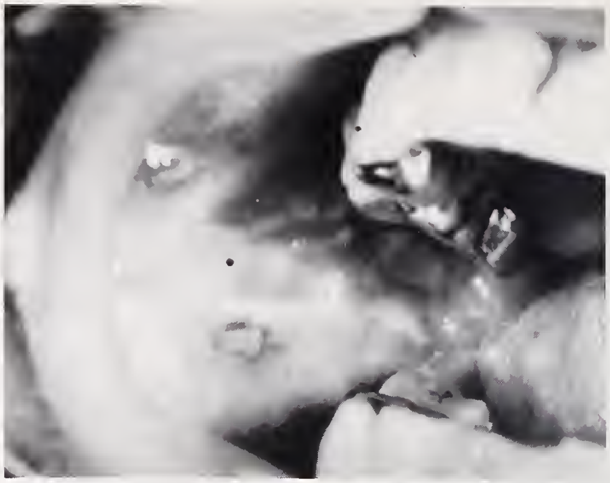

Fig. 1. Buccal mucosa mucositis with ulceration secondary to chemotherapy.

Scleroderma (Progressive Systemic Sclerosis)

Scleroderma is a chronic disease characterized by diffuse sclerosis of the skin, gastrointestinal tract, heart muscle, lungs, and kidney (22). The lips of a patient with scleroderma may appear to be pursed due to constriction of the mouth aperture, thus making it difficult to open the

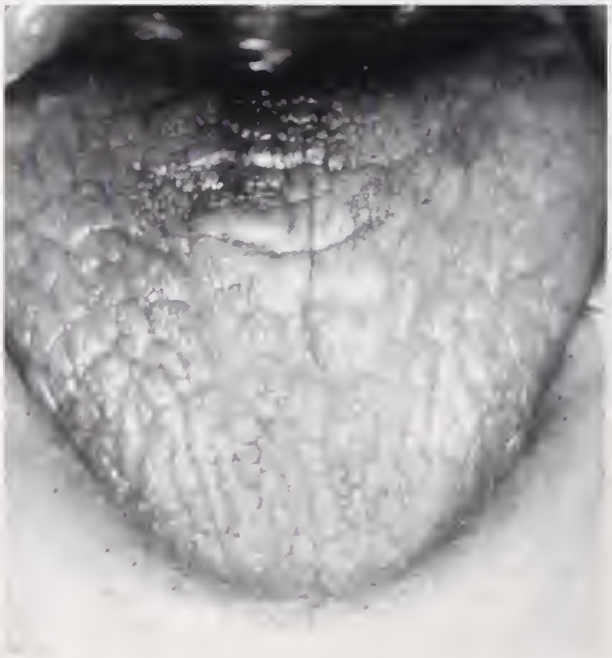

Fig. 2. Characteristic fissuring of tongue and loss of papillae as seen in xerostomia.

mouth. Stomatognathic function, denoting the mouth and jaws collectively, is also difficult. Esophageal fibrosis leads to lower esophageal sphincter hypotension and gastroesophageal reflux in 75% of scleroderma patients (23). Symptoms include dysphagia and heartburn. The oral mucosa appears pale and feels rigid. Multiple telangiectasias may be present (24). The tongue can lose mobility and become smooth in appearance as the palatal rugae flatten. Salivary hypofunction can also be present, although usually to a lesser degree than in Sjögren's syndrome. Radiographically, the periodontal ligament space is often thickened.

Lupus Erythematosus (LE)

Lupus erythematosus occurs as discoid lupus erythematosus (DLE) and systemic lupus erythematosus (SLE) (25). Oral lesions occur in 25–50% of patients with DLE versus 7–26% of patients with SLE (26, 27). In DLE, these lesions usually begin as an irregular whitish area that extends peripherally (Fig. 3). As they extend, the central area may become red and ulcerated while the border remains elevated and hyperkeratotic. Oral lesions of lichen planus are similar to those of DLE both clinically and histologically (28). Strict histologic criteria must be applied to distinguish one from the other (28).

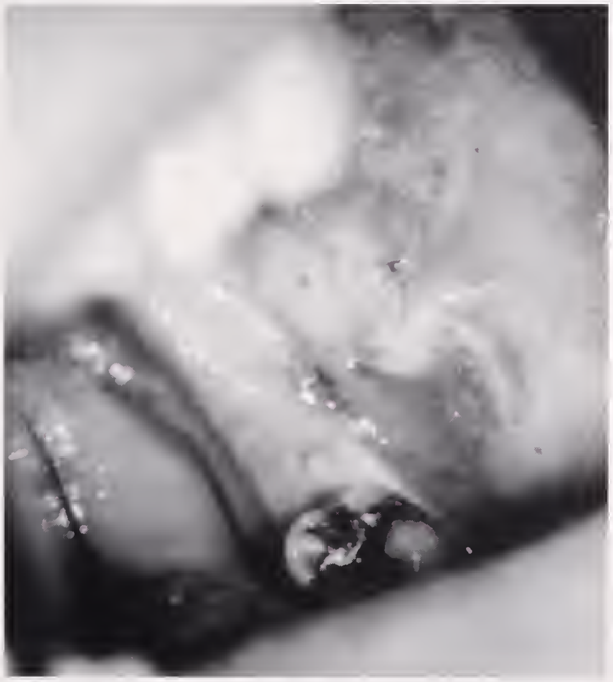

Fig. 3. Buccal mucosa exhibiting irregular white areas of ulceration as seen in discoid lupus erythematosus.

Oral or nasopharyngeal ulceration is recognized as a major diagnostic manifestation of SLE by the American Rheumatism Association Committee on Diagnostic and Therapeutic Criteria (29). These ulcerations are generally painless and often involve the palate (26). Purpuric lesions such as ecchymoses and petechiae may also occur. In up to 30% of patients with SLE, salivary gland involvement may occur concomitantly, leading to secondary Sjogren's syndrome and severe xerostomia (30).

Rheumatoid Arthritis

The temporomandibular joint (TMJ) is often involved in rheumatoid arthritis (31). This is usually characterized by erosions in the condyle leading to a decreased range of motion of the mandible with pain upon movement. Oral dryness and salivary gland swelling can also be found in patients with rheumatoid arthritis. These patients can also develop secondary Sjogren's syndrome (32). Limited jaw function may necessitate TMJ reconstruction once the active disease is controlled. Prosthetic joints may provide an interim solution for those patients severely affected with rheumatoid arthritis.

Oncological Diseases

Metastatic Disease

Oral metastatic tumors can occur in the soft or hard tissues (33). They are uncommon, however, comprising only about 1% of oral malignant neoplasms. Tumors metastasize more often to the jaws than to oral soft tissue. Tumors of the jaw are usually detected when the patient presents with swelling, pain, paresthesia, or when they spread to the soft tissue. Overall, the most common primary site for metastases to the jaw is the breast, whereas the lung is the most common primary site for oral soft tissue metastases (34). In men, the lung is the most common primary site for both jaw and soft tissue metastases. The molar region of the mandible is the most common bony site for metastasis. In about 30% of cases, the oral metastatic lesion is the first indication of an undiscovered malignancy (34).

The early manifestation of metastases to the attached gingiva may resemble one of three more common hyperplastic reactive lesions of the gingiva and must be evaluated by biopsy. The peripheral ossifying fibroma generally presents as a small, well-demarcated, focal mass with a sessile or pedunculated base on the free margin of the gingiva (Fig. 4). These pale pink to red lesions can be quite large (35) and can occur at any age (peak incidence is 20 years of age). The pyogenic tumor or "pregnancy tumor" which has a propensity to bleed, also occurs on the attached gingiva. This lesion is usually small (less than 1 cm in diameter), red, and ulcerated. Another small, well-demarcated, dark red focal mass attached to the gingiva with a sessile or pedunculated base is the peripheral giant cell granuloma (36). In summary, it is important to recognize that various tumors do metastasize rarely to the oral cavity.

Langerhans' Cell Histiocytosis (Histiocytosis X)

Langerhans' cell histiocytosis (LCH) represents a spectrum of clinical disorders ranging from a highly aggressive and frequently fatal leukemia-like disease affecting infants to a solitary lesion of bone (37). The presence of alveolar bone loss in young children with precocious exfoliation of primary teeth should suggest the possibility of LCH (38). LCH can also occur in adolescents and adults. Of the bones of the jaw, the mandible is the most frequently involved. The presenting signs usually include pain, swelling, ulceration, and loose teeth. Radiographically, the teeth often appear to be "floating in air" surrounded by large radiolucent regions. This is due to rapid alveolar bone loss. The term "eosinophilic granuloma of bone" is used when a solitary lesion is found, but multiple lesions may develop later (Fig. 5).

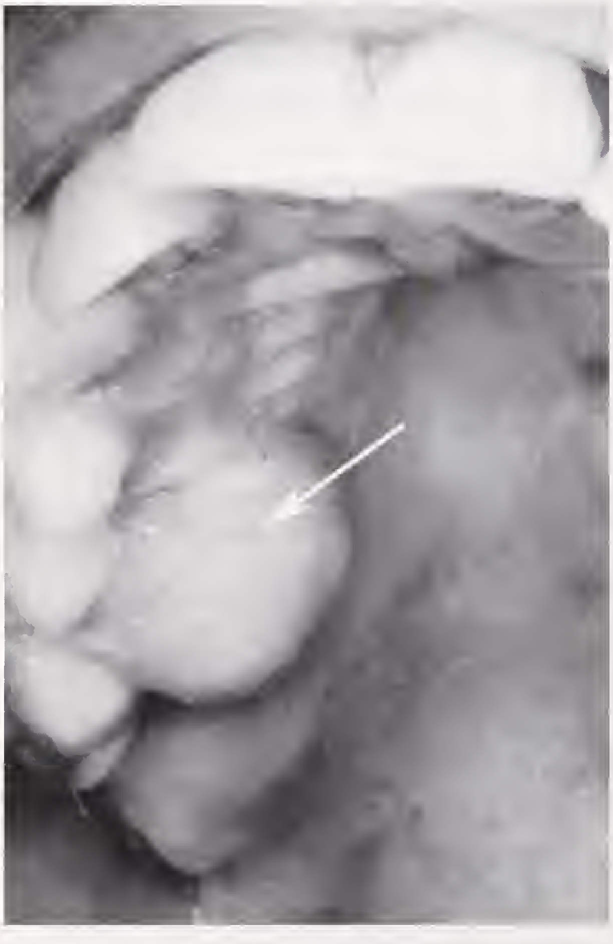

Fig. 4. Palatal peripheral ossifying fibroma originating between second maxillary premolar and first maxillary molar.

Endocrine Disorders

Diabetes Mellitus

There are many oral manifestations of diabetes mellitus, some having been described as early as 1862. Generally, the symptoms are more severe, and the rate of their progression appears to be more rapid in those patients who have poorly controlled insulin-dependent diabetes (IDDM) than in those who are well-controlled with insulin or in those with non-insulin-dependent diabetes (NIDDM). Studies indicate that age, duration of disease, and degree of metabolic control may play a more important role in the oral manifestations of diabetes rather than whether the type of disease is IDDM or NIDDM (39). About a third of diabetic patients complain of xerostomia, which may be due to an overall diminished flow of saliva and an increased salivary glucose level (40). Concomitant diffuse, nontender, bilateral enlargement of the parotid glands, called diabetic sialadenosis, may be seen in patients. This process may not be reversible, even after carbohydrate metabolism has been well controlled. Altered taste and burning mouth syndrome have also been reported in association with poorly controlled diabetes. Xerostomia may act as a predisposing factor in the development of oral infections. Dry and damaged mucosa is more susceptible to opportunistic infections by *Candida albicans* (40). Erythematous candidiasis presents as central papillary atrophy of the dorsal

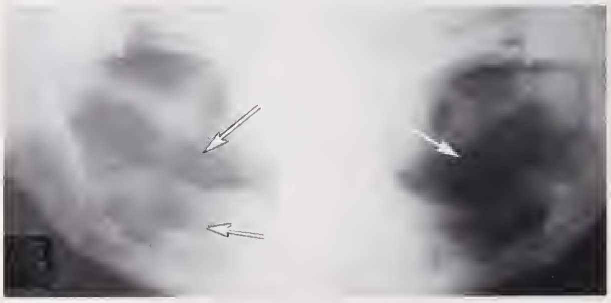

Fig. 5. Panoramic radiograph in this patient with Langerhans' cell histocytosis showing multiple radiolucencies of the mandible.

tongue papillae and has been reported in up to 30% of diabetic patients. Mucormycosis and benign migratory glossitis have also been shown to have a higher incidence in IDDM than in the general population.

It has been well documented that there is a higher incidence of dental caries in patients with poorly controlled diabetes. This has been attributed to increased glucose levels in the saliva and crevicular fluid (41). Poor healing, xerostomia with subsequent increased accumulation of plaque and food debris, higher susceptibility to infections, and pronounced hyperplasia of attached gingiva all contribute to the increased incidence of periodontal disease in diabetics (40).

Hypoparathyroidism

Decreased secretion of parathyroid hormone (PTH) may occur following iatrogenic surgical removal of the parathyroid glands, as well as from autoimmune destruction of the parathyroid tissue. Rare syndromes such as DiGeorge syndrome and the endocrine-candidiasis syndrome may be associated with this condition (42). Hypocalcemia follows the loss of parathyroid hormone. Chvostek's sign, a significant finding associated with hypocalcemia, is characterized by twitching of the upper lip when the facial nerve is tapped just below the zygomatic process. A positive response suggests a latent degree of tetany. If the hypoparathyroidism develops early in life, during odontogenesis or tooth development, a pitting enamel hypoplasia and failure of tooth eruption may occur (43). The presence of persistent oral candidiasis in a young patient may signal the onset of endocrine-candidiasis syndrome. Hypoparathyroidism may be only one of several endocrine deficiencies associated with this condition.

Hyperparathyroidism

Loss of the lamina dura surrounding the roots of the teeth is an early manifestation of hyperparathyroidism, with alterations in the jaw trabecular pattern characteristically developing next. There is a decrease in trabecular density, and blurring of the normal pattern produces a "ground glass" appearance on the radiograph (44). With persistent disease, other osseous lesions develop, such as the so-called "brown tumor" of hyperparathyroidism. The name of this lesion is derived from the color of the gross tissue specimen, which is usually dark reddish-brown due to the abundant hemorrhage and hemosiderin deposition within the tumor. Radiographically, these lesions are unilocular or multilocular well-demarcated radiolucencies which commonly affect the mandible, clavicle, ribs and pelvis. They may be solitary, but more often are multiple. The long-standing lesions may produce significant cortical expansion. Histologically, the lesion is characterized by a proliferation of extremely vascular granulation tissue which serves as a background for numerous multi-nucleated osteoclast-type giant cells. This is identical to another lesion known as the central giant cell lesion of the jaws.

Hypercortisolism

Hypercortisolism, or Cushing's syndrome, results from a sustained increase in blood glucocorticoid levels. This can be due either to corticosteroid therapy or to endogenous overproduction from the adrenal gland(s). Excess adrenocorticotrophic hormone (ACTH) from a pituitary tumor also causes hypercortisolism and Cushing's disease. Fatty tissue deposition in the facial area is described as the characteristic "moon" facies. (45). The patient may also present with a variable degree of facial hirsutism. Pathological fractures of the mandible, maxilla or alveolar bone may occur upon low impact trauma due to osteoporosis. Healing of fractures as well as healing of alveolar bone and soft tissues after dental extractions is also delayed.

Hypoadrenocorticism

Hypoadrenocorticism results from insufficient production of adrenal corticosteroid hormones caused by destruction of the adrenal cortex, a condition known as primary hypoadrenocorticism or Addison's disease. This is usually due to autoimmune destruction, but can also result from infection such as tuberculosis, metastatic tumor, amyloidosis, sarcoidosis or hemochromatosis. Secondary hypoadrenocorticism develops because of inadequate functioning of the pituitary gland. Orofacial manifestations include "bronzing" hyperpigmentation of skin, especially predominant in sun-exposed areas and over pressure points. This is caused by increased levels of beta-lipotropin or adrenocorticotrophic hormone (ACTH), both of which can stimulate melanocytes. These skin changes are often preceded by oral mucosal melanosis (46). The diffuse or patchy brown macular pigmentation most commonly occurs on the buccal mucosa, but can also occur on the floor of the mouth, ventral tongue, and other areas of the oral mucosa.

Renal Disease

Uremic Stomatitis

Uremic stomatitis has become relatively rare, seen mostly in cases of undiagnosed and untreated chronic renal failure. Painful plaques and crusts are distributed predominantly on the buccal mucosa, the floor or dorsum of the tongue, and the floor of the mouth. The incidence has decreased because of readily available dialysis centers throughout the country. The most commonly accepted mechanism behind the development of uremic stomatitis is irritation and chemical injury of mucosa by ammonia or ammonium compounds formed by the hydrolysis of urea in saliva by urease. This occurs when the intraoral concentration of urea exceeds 30 mmol/L (47). Hemorrhagic diathesis from inhibited platelet aggregation may also play a role due to local hemorrhage, resulting in decreased vitality and viability of the affected tissues, thus allowing bacterial infection.

There are two predominant types of uremic stomatitis (47). In Type I, there is a generalized or localized erythema of the oral mucosa and a thick gray pseudomembranous exudate which does not leave a bleeding or ulcerated base when removed. Additional findings may include pain, burning, xerostomia, halitosis, gingival bleeding, dysgeusia, or candidal infection. Type II leaves ulceration if the pseudomembranous film is removed. This type may indicate a more severe form of stomatitis, secondary infection, anemia or underlying systemic hematologic disturbances caused by renal failure. Histologically, both types of uremic stomatitis show evidence of an intense inflammatory process with heavy polymorphonuclear leukocytic infiltration and necrosis of the oral mucosa. Bacterial colonization is most commonly associated with *Fusobacterium*, spirochetes, or *Candida*.

Gastrointestinal Diseases

Crohn's Disease

In 1969, oral manifestations of Crohn's disease were first described as being identical to those occurring in the intestinal mucosa. Histologically, these lesions feature non-necrotizing granulomas in the submucosa, composed of multinucleated Langerhans' giant cells, epitheloid cells, lymphocytes, and plasma cells. These granulomas may be of various sizes and depths in the submucosa and their incidence varies from 10–99% (48, 49). Sometimes these granulomas can be found bulging into or within the lumina of lymphatics, a phenomenon known as "endovasal granulomatous lymphangitis" (49).

Clinically, these patients present with diffuse swelling of one or both lips with associated angular cheilitis, and "cobblestoning" of the buccal mucosa with hyperplastic rigid mucosa. Painful linear ulcerations in the buccal vestibule, painless localized swellings within the lips or face, tags attached to the buccal mucosa, fissuring on the midline of the lower lip, and erythematous edematous gingiva may be seen (50). The cervical lymph nodes may be firm and palpable as well. There is no direct time correlation between intestinal and oral lesions. Oral lesions have been documented to precede the intestinal lesions by years, and in some cases are the only manifestation of the disease. The oral lesions only respond to treatment with systemic steroids.

Ulcerative Colitis

Ulcerative colitis has been associated with destructive oral ulcerations resulting from immune-mediated vasculitis (51). These are similar to apthous ulcers, but appear less frequently than lesions of Crohn's disease. Pyostomatitis vegetans is believed to be an oral manifestation of ulcerative colitis. These multiple painless intraepithelial microabscesses connect in linear or serpentine tracks mostly on the mucosa of lips, soft palate and ventral tongue. Pyostomatitis gangrenosum is the extreme variant with large, long-lasting and destructive ulcers which cause significant tissue scarring (52).

Conclusion

Systemic diseases often present with abnormalities of the structures of the mouth and jaws. Proper diagnosis is essential to initiate the correct treatment. The primary care physician and dentist must be familiar with these problems if their patients are to be well served. We have outlined several of the more common clinical entities in this review article.

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Implant Reconstruction of the Jaws and Craniofacial Skeleton

Pamela L. Alberto, D.M.D.

Abstract

Full oral rehabilitation with a high degree of success is now possible with osseointegrated implants. Osseointegration is a direct connection between living bone and the titanium implant at the level of the light microscope. Osseointegrated implants are currently used to replace single teeth, support fixed bridges and stabilize full dentures. These implants can also be placed extraorally for attachment of facial prosthesis.

The surgical technique used to place implants intraorally into jaws or facial skeleton is performed in two stages using a local anesthetic and/or conscious sedation. During stage I surgery, holes are placed into the jaw using a series of gradually larger diameter burs until the desired diameter and depth of the bony preparation is achieved. The implant is then placed. The implant must remain undisturbed for 4 months for osseointegration to take place. Stage II surgery is then required to remove the mucosa over the implant and place the transmucosal abutment. After 1–2 weeks of healing, the restorative dentist can take an impression and fabricate the prosthesis.

On occasion, it is necessary to augment the height and width of the atrophic jaw with autogenous or allogeneic bone grafts prior to implant placement. Bone grafts are sometimes placed on the floor of the nose or the floor of the maxillary sinus.

Guided tissue regeneration is a technique used to generate bone within bony defects adjacent to implants.

With long-term rates of success (5 years) of 99% for implants placed in the mandible and 95% for those placed in the maxilla, reconstruction of the jaws and cranial facial skeleton with osseointegrated implants has become the treatment of choice. **Key Words:** Dental implant, osseointegration, implant reconstruction.

Introduction

For almost 200 years, implants have been placed in human jaws. Early types of dental implants came into common clinical use during the 1960s. At that time, there was little sound scientific research to characterize their success rates. In 1978, the National Institutes of Health (NIH) sponsored a conference which defined the criteria for implant success. The NIH implant success criteria are the following: (1) mobility of less than 1 mm in any direction; (2) bone loss no greater than one third of vertical height; (3) gingival inflammation amenable to treatment; and (4) implant to provide function for 5 years in 75% of the cases. At that time, none of the available implant systems met these criteria; thus, they were not accepted by the dental profession. In 1982, osseointegrated implants were first presented at an implant consensus development conference of the North American dental profession. "Osseointegration" is defined as a direct connection and attachment, as seen under light microscopy, between living bone and the load-bearing implant. This implant was reported to have a 91% success rate in the mandible for more than 15 years (1). Professor Per-Ingvar Branemark, an orthopedic surgeon, while studying bone healing and regeneration of the rabbit femur, using a titanium chamber, found serendipitously that bone adhered to the metal. Branemark performed extensive animal and clinical trials implanting various shapes and sizes of titanium implants into bone. In some cases, the

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From the Department of Oral and Maxillofacial Surgery, New Jersey Dental School, University of Medicine and Dentistry of New Jersey, Newark, NJ.

Address correspondence to Pamela L. Alberto, D.M.D., Clinical Associate Professor, Department of Oral and Maxillofacial Surgery, University of Medicine and Dentistry of New Jersey, New Jersey Dental School, 110 Bergen Street, Newark, NJ 07103-2400.

bone attached and adhered to the titanium so tightly that the bone fractured before it could be removed. He called this process of attachment and adherence "osseointegration" (Fig. 1).

The first clinical trials of these implants began in 1965 and continued thereafter for 17 years before marketing. Branemark found that what was needed to achieve osseointegration included a biocompatible material, atraumatic surgery with drilling temperatures below 47°C, precise adaptation of the implant to bone, and an undisturbed, immobile healing phase. Titanium was determined to be the most biocompatible material because a surface coating of titanium oxide created a stable ceramic interface onto which bone could be deposited (2). This oxide surface is a dynamic system and has been shown to release a measurable amount of titanium ion into the tissues. The reactivity of this oxide surface is one reason why titanium is so biocompatible. The implant surface and design can also influence osseointegration; a rough surface will increase the amount of contact surface. The design of the implant affects the transfer of functional load. It is imperative that the implant remain stable during the initial healing phase to prevent formation of connective tissue instead of bone. The quality and quantity of bone present also affect implant stability. Areas of the jaws with large amounts of cortical bone versus cancellous bone are advantageous for successful initial stabilization of the implant. A healing phase of 4 – 6 months in the jaws is necessary to achieve successful osseointegration.

Diagnosis and Treatment Plan

Proper treatment planning, along with implementation of appropriate surgical and prosthetic care, are the keys to implant success (3). A comprehensive medical evaluation is necessary to obtain information on systemic problems that may affect bone physiology and healing. Medical contraindications to implant placement include pregnancy, liver disease, endocrine disorders, and collagen/bone disease. Tobacco usage, poor oral hygiene and poor patient compliance are relative contraindications, especially if bone grafting is involved. A comprehensive intraoral physical exam is performed to assess the size of the ridge and the amount of attached gingiva. Impressions are taken of the maxilla and mandible, so surgical stents and radiographic stents can be fabricated. A 3-dimensional computed tomogram (CT) may be required to determine the available bone and obtain accurate measurements of bone height and width. Computer software is available to accurately plan the surgical treatment.

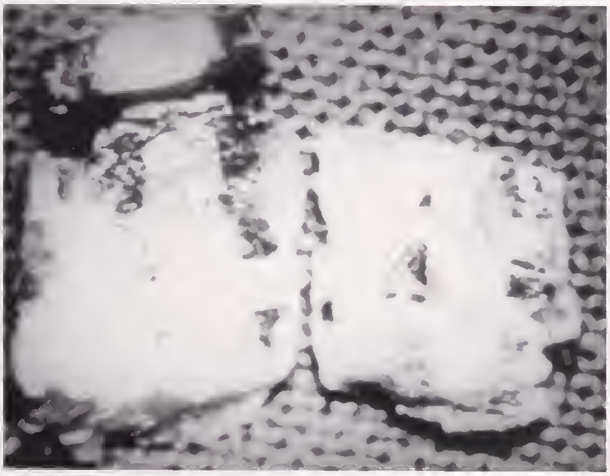

Fig. 1. Osseointegration: the direct connection between bone and titanium.

After the patient's attitude and expectations are evaluated, a treatment plan is developed noting the type of prosthesis, the number of implants, and the need for augmentation techniques. The surgical stent fabricated dictates the placement and angulation of the implants. This is critical to the final prosthetic result.

Types of Implants

There are 3 major subgroups of dental implants: subperiosteal, transosteal, and endosseous implants (Figs. 2a-c). A subperiosteal implant is a framework fabricated to fit intimately on top of the mandible or maxilla under the mucoperiosteum. The technique involves surgical exposure of the bone of the jaws, taking an impression of the bone, and making a model. The implant is then fabricated by the laboratory from this model. A second surgery is then required for placement of this subperiosteal implant. A model may first be fabricated from computer-aided design/computer-aided manufacturing (CAD/CAM) CT technology thereby eliminating the initial surgery to make the model. This implant has a 93% success rate over 5 years, but only a 50 – 60% success rate over 10 years.

The transosteal implant is an implant with a bone plate fitted against the inferior border of the symphysis. The bone plate is attached to the mandible by a series of penetrating posts. Two of these posts enter the oral cavity to support a lower denture. The technique requires an extraoral incision placed in the submental fold. An advantage is the ability to reattach the musculature and improve the cervicomental aesthetics. These implants have a 94% success rate over 5 years and an 87% success rate over 10 years (4).

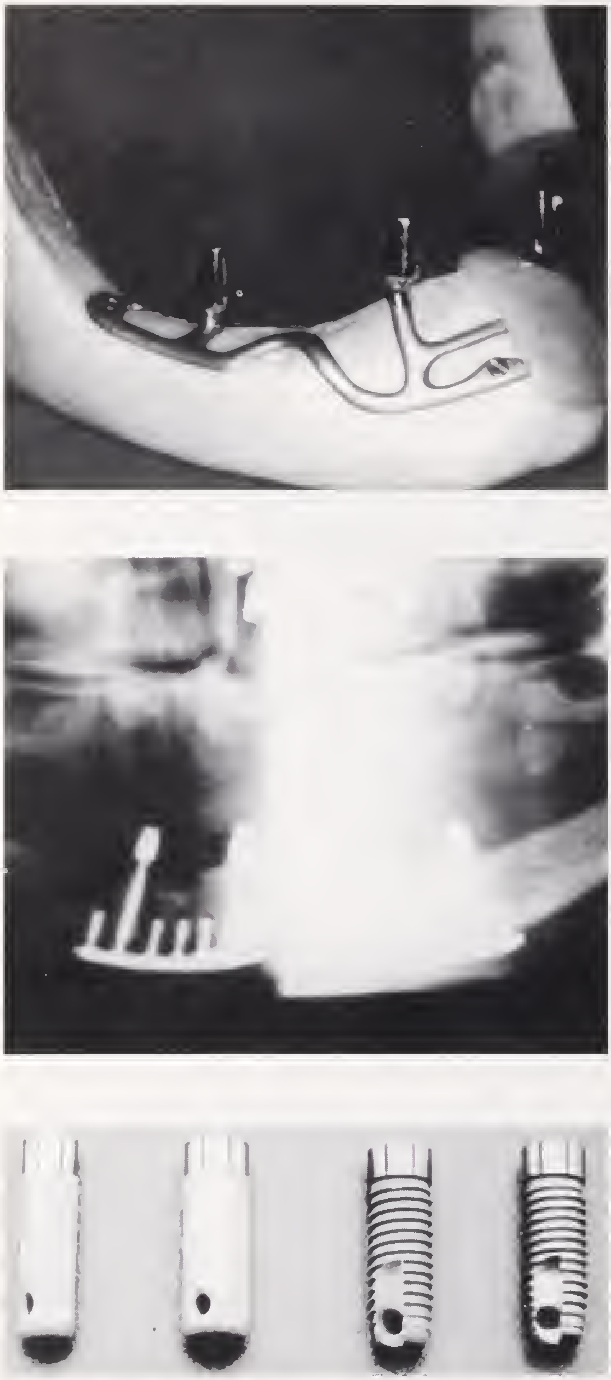

Fig. 2. a. Subperiosteal implant, b. transosteal implant, c. endosseous implant.

Endosseous implants are most frequently utilized. They are placed in the bone of the maxilla or mandible via intraoral incisions. There are several different designs available commercially, including screw, cylindrical, and blade types. They may be categorized as one- or two-stage implants. The one-stage implant is surgically placed in bone and projects through the mucosa into the oral cavity. The two-stage endosseous implants are placed in bone, and the mucosa is sutured over the implants to facilitate the healing phase. However, the healing phase is primarily dependent on the quality and quantity of bone. Usually, after 4–6 months, the implant is uncovered. An abutment which projects through the mucosa into the oral cavity is attached. The dentist can then complete the prosthetic restoration.

Surgical Technique to Achieve Osseointegration

The techniques for placing implants intraorally into the jaws or extraorally into the skull or facial bones are technically identical. This procedure is usually performed under intravenous sedation, but may be performed with local anesthetic nerve blocks. A surgical stent is placed in the mouth, identifying the position and angulation of implant placement. Using low speed (less than 1000 rpm) internally irrigated drill bits, holes are placed into the jaw. The depth and width of each hole are determined radiographically and are limited anatomically in relation to the position of the maxillary sinus and inferior alveolar nerve. These sites are then widened using a series of gradually larger burs. After the desired diameter and depth of the bony preparation is achieved, the implant is placed. Non-threaded cylinder implants are gently tapped into position, whereas threaded implants may require tapping the bony preparation prior to placing the implant (Fig. 3). After all the implants are placed, the mucoperiosteal flap is closed without tension, using a monofilament suture in an interrupted fashion. Postoperatively, a radiograph is taken to assess the position of the implants. The patient is then placed on analgesics and on a 0.12% chlorhexidine gluconate rinse for 2 weeks. At that time, the patient is allowed to begin a soft diet and given an interim prosthesis. After completion of stage I surgery, the patient is followed on a monthly basis for the entire healing period. Once the healing period is completed, stage II surgery is required to uncover the implant and place the transmucosal abutment. It takes approximately one to two weeks for the mucosa to heal around the abutment before the dentist can take an impression and fabricate a prosthesis. There are multiple restorative options, including single crowns, fixed bridges, and implant retained removable dentures.

Advanced Surgical Techniques

In the severely atrophic mandible, it may be necessary to augment the height and width with

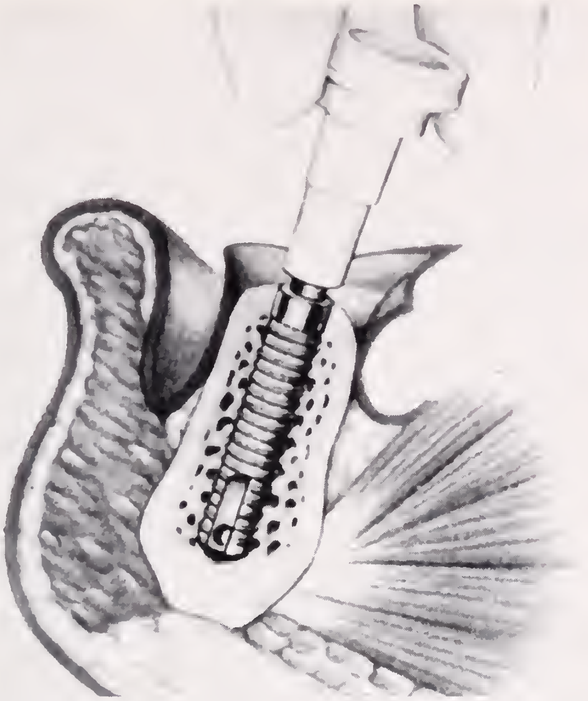

Fig. 3. Implant placement.

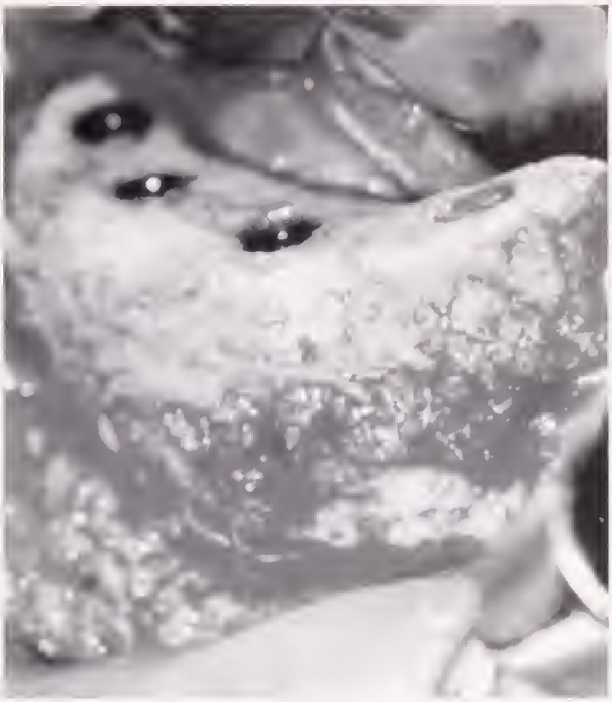

Fig. 4. Mandibular graft with endosseous implants.

autogenous grafts prior to implant placement (5). Occasionally, implants can be placed concomitantly with the graft and used to assist in the stabilization of the graft. The donor sites used for autogenous grafts include the iliac crest, tibia, mandibular symphysis and mandibular lateral ramus. The site selected is determined by the amount of graft needed for the augmentation. Allogeneic bone grafts can be used alone or in conjunction with autogenous bone. An augmentation technique can be utilized to fashion a one-piece autogenous graft, harvested from the inner table of iliac crest, to conform to the mandible. The graft is then rigidly fixed to the mandible with 4–6 screw-type endosseous implants (Fig. 4). This technique obviates the need for placement of the implants after consolidation of the graft, thereby reducing treatment time by 3–6 months. If inadequate bone height is present in the posterior mandible, a procedure may be used to reposition the mandibular nerve so that implants can be placed medial to the lateralized nerve. This procedure carries the risk of permanent paresthesia, so it is not widely used. Immediate implant placements with an onlay graft in the mandible have a 97% success rate.

In the severely atrophic maxilla, the problems of implant reconstruction and types of grafts are different. The maxilla has a limited amount of bone mass due to the maxillary sinus and nasal cavity. The bone quality is less dense due to the large amount of cancellous bone and thin cortical plates. Thus, the onlay graft rigidly fixed to the maxilla with endosseous implants has an 87% success rate. Bone grafts can also be placed on the floor of the nose or the floor of the maxillary sinus. A common procedure used to increase the amount of bone in the posterior maxilla is the sinus lift procedure (6) (Fig. 5). A window is cut into the lateral wall of the sinus without tearing the schneiderian membrane of the maxillary antrum. The lateral window is elevated superiorly along with the membrane off the bony walls using various sized curettes. The lateral wall now becomes the new floor of the sinus. A particulate bone graft material, allogeneic, autogenous, or a combination thereof, can then be placed. This procedure has a success rate of about 90% (7), especially when autogenous bone is used. Implants may be placed at the same time as the graft procedure, provided there is more than 5 mm of residual alveolar ridge to stabilize the implants. The floor of the nose graft is constructed via a vestibular incision with elevation of the anterior nasal floor. A corticocancellous graft is placed and can be stabilized using the endosseous implants or small 1.5 mm titanium screws.

"Guided tissue regeneration" is a procedure used to regenerate bone within bony defects and adjacent to implants. When a barrier is placed between a bony defect and the overlying epithelium, bone growth will preferentially take place and fill the defect. This barrier or membrane selectively prevents the ingrowth of connective

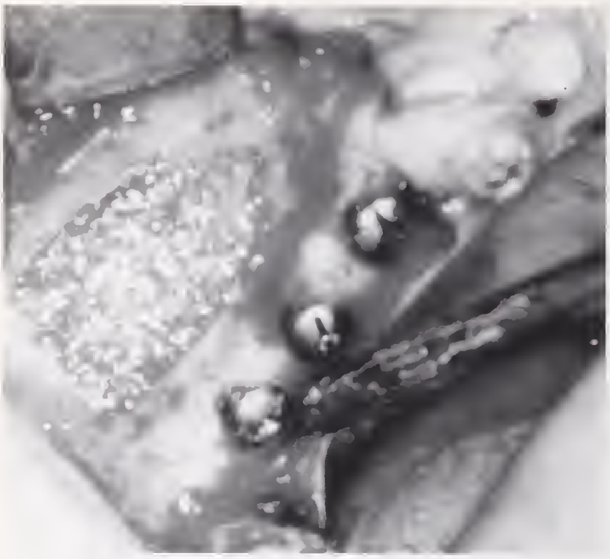

Fig. 5. Sinus lift procedure with implant placement.

tissue and epithelium. Various materials can be used as barriers, but expanded polytetrafluoroethylene has been the most extensively tested and utilized. The procedure is particularly useful in grafting bony defects around implants and increasing the width of the bony ridge in preparation for implants.

Clinical Applications

Osseointegrated implants are used to replace single teeth, to support fixed bridges, and to stabilize removable full dentures. These implants, which offer full oral rehabilitation with a high degree of success, may also be placed extraorally for the attachment of facial prosthetic devices (8, 9). Historically, these structures have been restored using a silicone prosthesis attached to the face with tissue adhesives, but these adhesives are not stable when heat or moisture is present. Using osseointegrated implants to anchor the prosthesis more securely allows these patients better function.

Implants also can be used to anchor bone conduction hearing aids (10). The implant is placed directly into the mastoid process. The conduction of sound to the auditory nerves directly through bone improves the level of hearing by 15 decibels. When combined with an auricular prosthetic device, function can be further enhanced along with improved cosmetic appearance. Osseointegrated implants have been used to rigidly fixate proximal and distal stems of the joint (11) and to reconstruct metacarpophalangeal and proximal interphalangeal joints in the hand.

Success and Failure

The criteria of success for endosseous implants are as follows: the implant should not demonstrate any clinical mobility, associated symptoms of pain or discomfort, damage to adjacent structures and crestal bone loss. If an implant becomes mobile, it should be removed. Long-term success rates with intraoral and extraoral implants have been established. A 5-year success rate of 99% has been achieved for implants placed in the mandible; for those placed in the maxilla, the rate is 95%. The 5-year success rate of 93% has been achieved with extraoral implants in patients with no previous radiation therapy (12).

The common causes of implant failure are biomechanical overload and infection. Bacterial toxins and compressive forces will cause tissue changes leading to epithelial downgrowth, bone resorption, mobility and implant failure. Biologic, iatrogenic, or mechanical factors can also lead to complications and failure. Poor bone quantity and/or quality, smoking, and previous radiation are the biologic factor; inappropriate planning, patient selection, and surgical and prosthetic treatment are the iatrogenic factors; and patient parafunctional habits and forceful manipulation are the mechanical factors that may contribute to failure and complications. Fortunately, most of these complications are treatable with early detection through careful follow-up during the healing period.

Conclusion

Osseointegrated implants have made possible the reconstruction of the jaws and cranial skeleton, and is the treatment of choice. Long-term success rates exceed 93%, and even this degree of success may be subject to improvement, if and when new materials become available. .

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Oral Manifestations of HIV Disease

David A. Sirois, D.M.D., Ph.D.

Abstract

Oral disease is frequently associated with HIV. While nearly all oral disorders associated with HIV infection also occur in other conditions characterized by immunosuppression, no other condition is associated with as wide and significant a spectrum of oral disease as is HIV infection. Many HIV-associated oral disorders occur early in HIV infection, not infrequently as the presenting sign or symptom. Thus, early detection of associated oral disease should, in many cases, result in earlier diagnosis of HIV infection. Likewise, awareness of the variety of oral disorders which can develop throughout the course of HIV infection, and coordination of health care services between physician and dentist, should improve overall health and comfort of the patient. This paper reviews the clinical, diagnostic and therapeutic aspects of HIV-associated oral disorders. **Key Words:** HIV, oral manifestations, oral disease.

Introduction

Based on standard classification and diagnostic criteria, common HIV-associated oral disorders can be broadly classified into four categories by pathophysiological process: infection (fungal, viral, bacterial), neoplasm, immune-mediated, and other (xerostomia, pain syndromes, and nutritional) (1) (Table 1). No particular oral lesion is uniquely associated with HIV infection. However, the presence of one or more lesions requires that HIV infection be considered as a possible underlying cause. Some oral lesions, such as oral candidiasis and oral hairy leukoplakia, are so strongly associated with HIV infection that they have been incorporated into the Centers for Disease Control and Prevention clinical classification of HIV disease (2). Indeed, the emergence of one or more oral lesions correlates highly with HIV progression. A CD4 lymphocyte count of less than 200/mm^3 is a reliable prognosticator of active disease and probability of shortened lifespan (3–8). The spectrum of HIV-associated oral lesions also varies with transmission risk-factor, gender, age, and health-care access (7–13).

Oral health is an important component of overall health status in HIV infection. Even common dental diseases such as caries and periodontal disease have greater impact on patients with HIV infection. Odontogenic pain and non-replacement of missing teeth may limit oral intake of food required for adequate nutrition. Many medications used to treat HIV infection and associated opportunistic infections contribute to increased numbers of caries as a result of decreased salivation and cariogenic fermentable carbohydrate substrates in the presence of several topical oral medications. Painful HIV-associated oral diseases such as necrotizing ulcerative periodontitis and stomatitis, major aphthous ulceration, candidiasis, and Kaposi's sarcoma impair ingestion of food and negatively impact on nutritional health. Therefore, it is essential that the physician and dentist, together, identify and reduce risk factors for oral disease in the patient with HIV infection.

From the Division of Oral Medicine, Department of Oral Pathology, Biology, and Diagnostic Sciences, New Jersey Dental School, University of Medicine and Dentistry of New Jersey, Newark, NJ.

Address correspondence to David A. Sirois, D.M.D., Ph.D., Associate Professor and Director, Division of Oral Medicine, Department of Oral Pathology, Biology, and Diagnostic Sciences, University of Medicine and Dentistry of New Jersey, New Jersey Dental School, 110 Bergen Street, Room C-829, Newark, NJ 07103.

TABLE 1
Common HIV-Associated Oral Disorders

INFECTION
- **Fungal:** candidiasis; cryptococcus; histoplasmosis; aspergillosis.
- **Viral:** herpes simplex virus; oral hairy leukoplakia (Epstein-Barr); human papilloma virus; cytomegalovirus.
- **Bacterial:** bacillary angiomatosis *(Rochalimaea henselae)*; linear erythematous gingivitis; necrotizing ulcerative periodontitis; syphilis *(Treponema pallidum)*.

NEOPLASM:
- **Fungal:** Kaposi's sarcoma.
- **Viral:** non-Hodgkin's lymphoma.

IMMUNE-MEDIATED:
- **Fungal:** major aphthous.
- **Viral:** necrotizing stomatitis.

OTHER:
- **Fungal:** xerostomia; parotid disease.
- **Viral:** pain syndromes.
- **Bacterial:** nutritional.

Fungal Infections

Candidiasis. The most common HIV-related oral lesion is candidiasis, predominantly due to *Candida albicans*. While *Candida* can be isolated from 30–50% of the oral cavities of healthy adults, making it a constituent of the normal oral flora, clinical oral candidiasis rarely occurs in healthy patients (13, 14). In stark contrast, clinical oral candidiasis has been reported to occur in 17–43% of patients with HIV infection and in more than 90% of patients with AIDS (9, 15–18). One report found that unexplained oral candidiasis in healthy adults with risk factors for HIV infection predicted the development of clinical signs of AIDS within 3 months (19).

Based on clinical appearance, oral candidiasis can appear as one of four distinct clinical entities: erythematous or atrophic candidiasis, pseudomembranous candidiasis, hyperplastic or chronic candidiasis, and angular cheilitis. In all cases, the infection is superficial. While in most instances the clinical appearance is adequate to arrive at a diagnosis, simple exfoliative cytology will identify the characteristic budding yeast and hyphae when the clinical diagnosis is uncertain. The appearance of each clinical type of candidiasis is summarized in Table 2 and representative photographs are depicted in Fig. 1.

Treatment of oral candidiasis is determined by the clinical type, distribution, and severity of infection (20). Topical treatment is effective for limited and accessible lesions. Clotrimazole troches, nystatin pastilles, and nystatin oral suspension are effective for mild-to-moderate erythematous and pseudomembranous candidiasis. However, prolonged use of these agents can result in significant dental caries due to the fermentable carbohydrate substrates. Increased risk of caries can be avoided by using nystatin oral suspension (100,000 units/5 ml, rinsing mouth and expectorating 3 times/day). Also clotrimazole vaginal troches can be effective (patient dissolves 1 troche in the mouth 3 times daily). Chlorhexidine 0.12% oral rinses do not contain a cariogenic substrate (21, 22) and may be similarly effective. Topical amphotericin B is also a useful non-cariogenic treatment for resistant candidiasis and can be prepared by dissolving 50 mg in 500 ml of sterile saline (0.1 mg/ml). Regular, gentle mechanical debridement with soft gauze soaked with 10% povidone-iodine or chlorhexidine 0.12% solution is very effective in managing focal, limited candidiasis. Clotrimazole 1% cream, miconazole or ketoconazole 2% cream, nystatin ointment, and Vytone® (hydrocortisone iodoquinol) 1% cream are useful medications for

TABLE 2
Clinical Types of Oral Candidiasis

Type	Appearance	Significance
Erythematous/Atrophic	erythematous or atrophic macular patches on palate, buccal mucosa, and tongue	associated with early HIV disease
Pseudomembranous	yellow-white plaque which can be easily wiped away; any mucosal surface	associated with initial and progressive immune deterioration; CD4<400
Hyperplastic/ Chronic	white non-removable plaque; may be stained by food; any mucosal surface	associated with severe immune suppression / long-standing HIV disease; may be associated with increased risk for carcinoma.
Angular Cheilitis	radiating fissures from labial commissure, sometimes covered with a pseudomembrane	can occur during all stages of HIV disease; xerostomia may be a contributing factor.

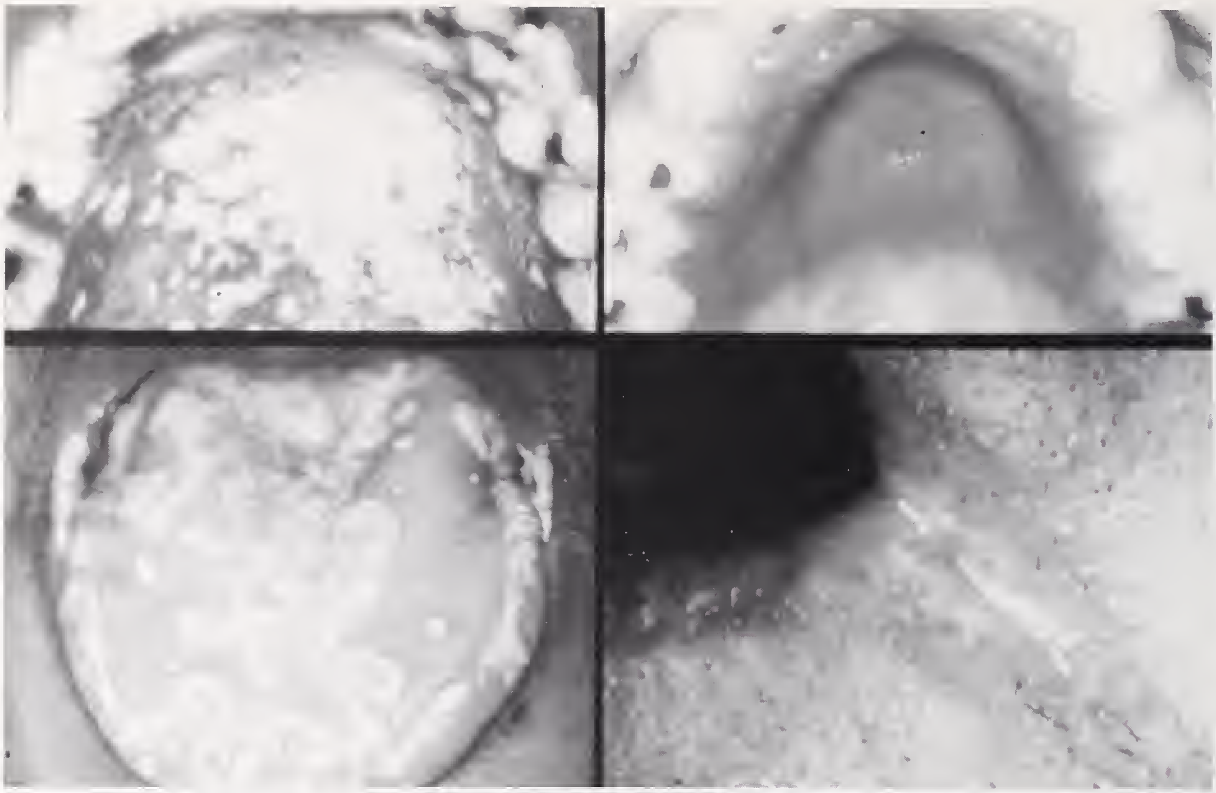

Fig. 1. Clinical spectrum of oral candidiasis. Upper left: pseudomembranous, removable white plaques; upper right: erythematous or atrophic candidiasis; lower left: hyperplastic (non-removable) candidiasis; lower right: angular cheilitis.

angular cheilitis and for application to a removable denture base when there is candidal infection involving the underlying mucosa. When long-term treatment or prophylactic use is required, systemic antifungal therapy is often required.

Systemic treatment for oral candidiasis involves the use of imidazole (ketoconazole) and triazole (fluconazole and itraconazole) antifungal medications. Ketoconazole is hepatotoxic and requires gastric acid for absorption, thereby limiting its usefulness in patients with HIV infection who may also have developed gastric achlorhydria. Fluconazole is an excellent systemic antifungal medication with a favorable therapeutic index, making it the preferred systemic antifungal medication. Itraconazole (100 mg tablet, 1–2 tabs/day) is another excellent systemic antifungal for use alone, or in combination with fluconazole (100 mg tablet, 1–2 tabs/day), for resistant candidiasis. Therapy should be continued for 2 weeks, until clinical infection is eliminated. Prophylaxis should be reserved only for those patients with demonstrated recurrence who cannot be controlled with topical medication. Treatment strategies and doses for antifungal therapy are summarized in Table 3.

Deep Fungal Infections. Unlike the superficial infection of candidiasis, several systemic fungal infections can infrequently lead to single or multiple, deep oral lesions with the potential for considerable local tissue destruction. Cryptococcosis, histoplasmosis, aspergillosis, and mucormycosis are uncommon oral deep fungal infections which require histological diagnosis. Treatment typically requires the use of intravenous antifungal therapy with amphotericin.

Viral Infections

Herpesvirus accounts for the majority of HIV-related oral viral infections, most frequently as recurrent oral herpes due to herpes simplex virus (HSV) or Epstein-Barr virus (EBV)-induced oral hairy leukoplakia (OHL) (23). Less commonly occurring viral infections involving the oral cavity include cytomegalovirus and human papilloma virus. Figs. 2 and 3 illustrate the common oral viral infections which occur most frequently in patients with HIV infection.

Herpes Simplex Virus. Intraoral herpes in healthy individuals results in multiple, small, shallow ulcerations with irregular raised white borders. Small clusters of lesions usually coa-

TABLE 3

Treatment Strategies and Doses for Antifungal Therapy

Drug	Route	Indication	Dose	Notes
clotrimazole troche	topical	erythematous and pseudomembranous	10 mg troche, 1 troche 5 times daily	cariogenic
nystatin pastille	topical	erythematous and pseudomembranous	200,000 U, 1 pastille 4–5 times daily	cariogenic
nystatin suspension	topical	erythematous and pseudomembranous	500,000 U/5 cc, 1 tsp rinse and swallow 4 times daily	cariogenic
nystatin vaginal troche	topical	erythematous and pseudomembranous	100,000 U, 1 troche 5–6 times daily	non-cariogenic
chlorhexidine 0.12%	topical	erythematous and pseudomembranous	1 tsp rinse and spit out 3 times daily; debridement with soaked gauze	
clotrimazole 1% cream miconazole 2% cream ketoconazole 2% cream hydrocortisone-iodoquinol 1% cream	topical	erythematous and pseudomembranous; angular cheilitis	apply to affected area 4 times daily	
ketoconazole	systemic, oral	erythematous and pseudomembranous	200 mg tab, 2 stat, 1 tab daily	hepatotoxic, take after meals
fluconazole	systemic, oral	all intra-oral types	100 mg tab, 2 stat, 1 tab 1–2 times daily	
itraconazole	systemic, oral	fluconazole resistance	100 mg cap, 2 caps once daily after a full meal	
amphotericin B	topical, systemic IV	fluconazole and itraconazole resistant	50 mg in 500 cc DW, rinse and spit out 3 times daily	

lesce to form a larger ulcer, which heals uneventfully in 7–10 days. While the prevalence of seropositive HSV and the rate of reactivation is similar among both HIV-infected and non-infected populations, estimated to be 60% for those older than 30 years of age, recurrent intraoral HSV in patients with HIV infection often results in ulceration and pain of longer duration (24–27). Recurrent intraoral HSV lesions occur more commonly on poorly keratinized tissue like the buccal and labial mucosa, an uncommon site in healthy individuals. The pain associated with persistent herpetic ulceration can result in reduced oral intake of food and significant weight loss. Clinical diagnosis can be assisted by culture and examination of a cytologic smear for the virus. Culture results should be interpreted with caution due to the high HSV seropositivity and the potential for false negative results due to silent shedding of HSV.

Intraoral HSV infection responds well to systemic acyclovir, 2 grams daily in divided doses. However, the incidence of acyclovir-resistant HSV has increased among patients with HIV infection. For most of these cases, oral famciclovir and valacyclovir and intravenous foscarnet alone or in combination are effective. Topical acyclovir is approved for genital HSV infections, but has been found to have little therapeutic effect for oral HSV (28–31). Although penciclovir was recently released as the first topical antiviral medication indicated for treatment of herpes simplex labialis, it reduced time to healing by only 0.7 days in healthy individuals compared to vehicle placebo, and no data on its use in patients with HIV infection are available (32).

Oral Hairy Leukoplakia (OHL). Although originally postulated to be pathognomonic for HIV infection (33), this lesion has subsequently been reported in other immune deficiency states as well as in immunocompetent individuals (34, 35). It appears as an asymptomatic adherent white patch with vertical corrugations, most commonly on the lateral borders of the tongue (Fig. 3). It may infrequently be confused with hypertrophic candidiasis and is predominantly found in

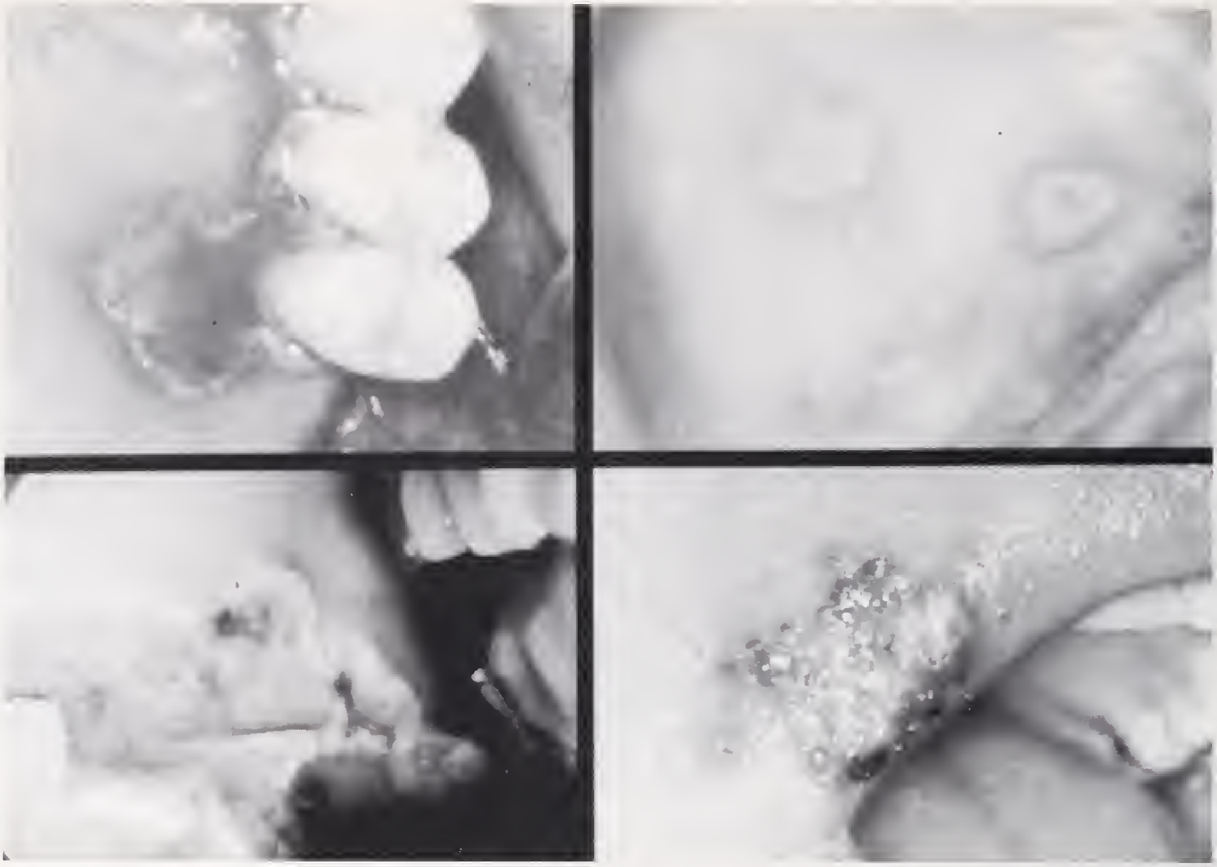

Fig. 2. Oral HSV lesions in patients with HIV infection. Upper left, right: coalesced shallow palatal HSV ulceration; lower left: extensive chronic HSV on the buccal mucosa; lower right: HSV labialis of 4 weeks' duration.

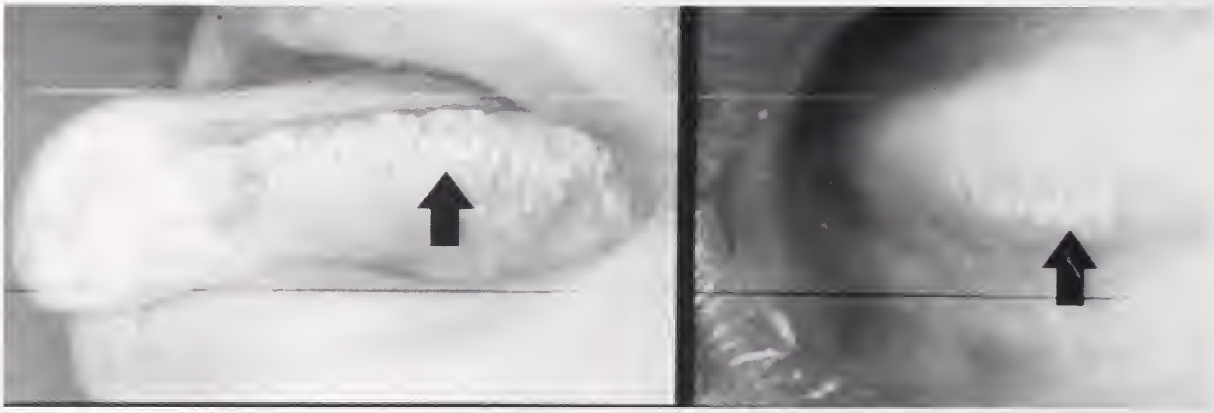

Fig. 3. Oral hairy leukoplakia due to Epstein-Barr virus. Adherent white corrugated patches on the lateral tongue.

homosexual males. Oral hairy leukoplakia has since been shown to be associated with a localized Epstein-Barr virus (EBV) infection and occurs most commonly in individuals whose CD4 lymphocyte count is less than 200/mm^3 (5). While the diagnosis is most often clinical, histological inspection will reveal typical epithelial hyperplasia suggestive of EBV infection. This asymptomatic lesion does not require treatment. However, for cosmetic purposes, some patients may request treatment. Oral acyclovir (3,200 mg daily in divided doses), topical podophyllum resin, retinoids, and surgical removal have all been reported as successful treatments. In most cases, the lesion returns after initial therapy, thus requiring prophylactic treatment with acyclovir 200 mg daily (36–38).

Human Papilloma Virus (HPV). In some patients with HIV infection, HPV causes a focal epithelial and connective tissue hyperplasia, forming an oral wart (Fig. 4). More than 50 strains of HPV exist. The most common genotypes found in the mouth of patients with HIV infection are 2, 6, 11, 13, 16 and 32. Surgical removal, with or without intraoperative irrigation with podophyllum resin, is the treatment of choice.

Cytomegalovirus (CMV). It is necessary to recognize oral CMV, which is an uncommon cause of intraoral ulceration in patients with HIV disease. Such a lesion may represent an early sign of disseminated CMV infection (39). Disseminated CMV infection must be diagnosed as early as possible because of the serious nature of its sequelae, including retinitis and meningitis. CMV has been detected postmortem in one or more organ systems in as many as 90% of patients with AIDS (40). Oral CMV infection typically appears as a solitary, chronic deep ulceration most often involving the buccal and labial mucosa. Clinically, it is indistinguishable from other nonspecific ulcerations such as chronic HSV and major aphthous ulceration. Thus, biopsy and histological inspections are essential for definitive diagnosis. Ganciclovir is the drug of choice, now available in a tablet formulation. Many patients with a history of CMV are placed on a prophylactic regimen (1.0 g ganciclovir, 3 times daily with food).

Bacterial Infections

Although isolated cases of oral infection with *Klebsiella pneumoniae, Enterobacter cloacae, Actinomyces israelii, Escherichia coli,* and *Mycobacterium avium intracellulare* have been reported in patients with HIV infection, the most common oral lesions associated with bacterial infection are linear erythematous gingivitis, necrotizing ulcerative periodontitis, and, much less commonly, bacillary epithelioid angiomatosis and syphilis. In the case of the periodontal infections, the bacterial flora is no different from that of a healthy individual with periodontal disease. Thus, the clinical lesion is a manifestation of the altered immune response to the pathogens.

Linear Erythematous Gingivitis. This entity appears as a 1–3 mm band of marginal gingival erythema, often with petechiae (Fig. 5). It is typically associated with no symptoms or only mild gingival bleeding and mild pain. Histological examination fails to reveal any significant inflammatory response, suggesting that the lesions represent an incomplete (aborted) inflammatory response, principally with only hyperemia present. There is no evidence to suggest that this entity will proceed to the far more destructive necrotizing periodontitis. Unlike conventional gingivitis, the erythema often persists following simple dental prophylaxis. Oral rinsing with chlorhexidine gluconate 0.12% often reduces or eliminates the erythema and typically requires prophylactic use to avoid recurrence.

Necrotizing Ulcerative Periodontitis (NUP). This unique periodontal lesion is characterized by generalized deep osseous pain, significant erythema that is often associated with spontaneous bleeding, and rapidly progressive destruction of the periodontal attachment and bone (Fig. 5). The destruction is not self-limiting and can result in loss of the entire alveolar process in the involved area. This very painful associated lesion adversely affects oral intake of food, resulting in significant and rapid weight loss. Because the periodontal microflora is no different from that seen in healthy patients, the lesion probably results from the altered immune response in HIV infection. More than 95% of patients with NUP have a CD4 lymphocyte count of less than

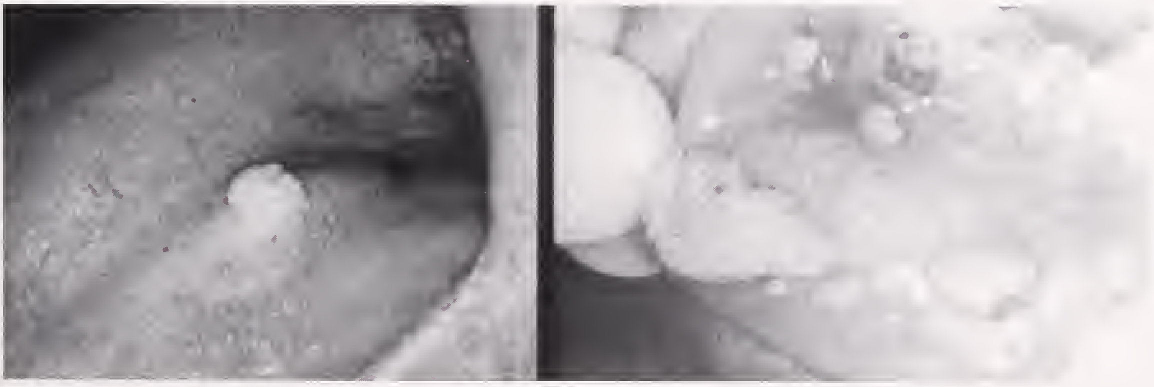

Fig. 4. Human papilloma virus. Solitary oral wart (left) and multiple, recurrent warts (right).

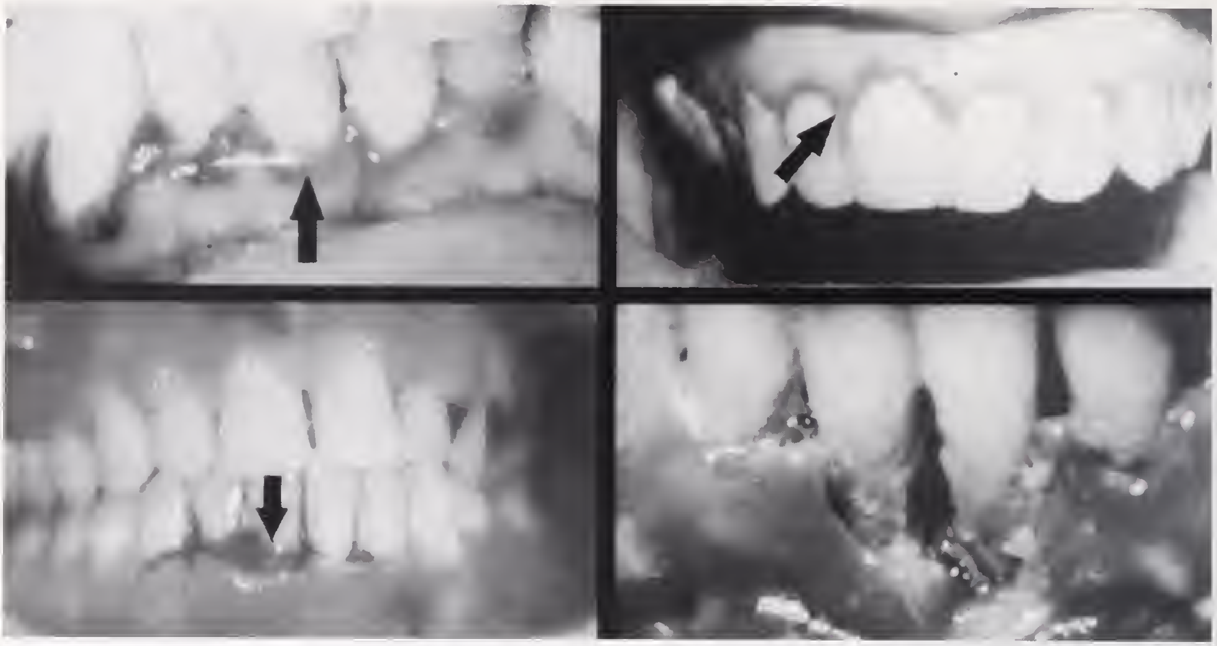

Fig. 5. Periodontal disease in HIV infection. Upper left, right: Linear erythematous gingivitis; lower left, right: Necrotizing ulcerative periodontitis.

200/mm^3 (41, 42). Treatment consists of rinsing twice daily with chlorhexidine gluconate 0.12%, metronidazole (250 mg orally four times daily for 10 days) and periodontal debridement, which is performed after antibiotic therapy has been initiated. Within 36–48 hours of antibiotic therapy, relief of pain associated with NUP is obtained.

Bacillary Epithelioid Angiomatosis (BEA). This recently described lesion appears to be unique to HIV infection and is often clinically indistinguishable from oral Kaposi's sarcoma (KS) (43, 44). Since both may present as an erythematous, soft mass which may bleed upon gentle manipulation, biopsy and histological examination are required to distinguish BEA from KS. The presumed etiological pathogen, *Rochalimaea henselae,* can be identified using Warthin-Starry staining. Both KS and BEA are histologically characterized by atypical vascular channels, extravasated red blood cells, and inflammatory cells. However, prominent spindle cells and mitotic figures occur only in KS. Erythromycin (erythromycin estolate, 500 mg 4 times daily for at least 10 days) is the treatment of choice for BEA.

Syphilis. While the prevalence of syphilis infection has risen significantly over the past decade, it is an uncommon cause of intraoral ulceration, even in HIV infection. Its appearance is no different from that observed in healthy individuals: it is a chronic, nonhealing, deep, solitary ulceration; often clinically indistinguishable from that due to tuberculosis, deep fungal infection, or malignancy. Dark field examination may demonstrate treponema. Positive reactive plasma reagin (RPR) and histological demonstration of *Treponema pallidum* is diagnostic. Patients with newly diagnosed syphilis should be referred to their physicians for evaluation and treatment; combination treatment with penicillin, erythromycin and tetracycline is the treatment of choice, the dosage and duration of treatment depending on presence or absence of neurosyphilis (56).

Neoplasms

Kaposi's sarcoma is the most common intraoral malignancy associated with HIV infection. Recognition of the lesion is essential, since oral KS is often the first manifestation of the disease and is a diagnostic criterion for AIDS (2). The lesion may appear as a red-purple macule, an ulcer, or as a nodule or mass. Intraoral KS occurs on the heavily keratinized mucosa, the palate being the site in more than 90% of reported cases (Fig. 6). However, lesions have also been reported on the gingivae, tongue, and buccal mucosa. The skin should also be examined for lesions whenever oral lesions are discovered. KS is especially common among homosexual and bisexual males and is rarely found in HIV-infected women (9, 10, 45). A new human herpes virus (HHV8) has recently been demonstrated to be an important cofactor in the development of

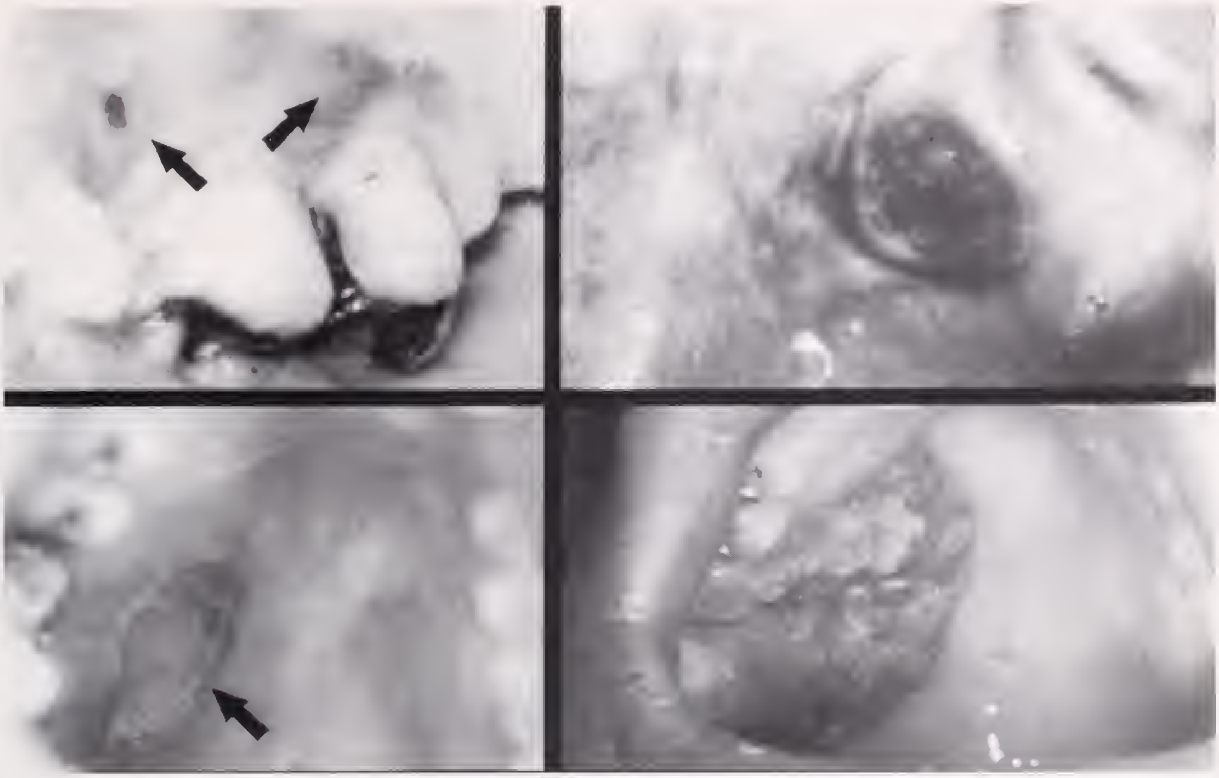

Fig. 6. Intraoral Kaposi's sarcoma. Variety of appearance: macule (upper left), plaque (upper right), ulcer (lower left), and mass (lower right).

KS, and prophylaxis with foscarnet and ganciclovir, but not acyclovir, has been shown to reduce the incidence of KS in a large at-risk cohort (46, 47).

Definitive diagnosis of KS requires histological examination. There is no cure for KS. Therapy for intraoral KS should be instituted at the earliest sign of the lesion, the goal being local control of the size and number of lesions. When only one or a few lesions exist and the lesions are small (<1 cm), intralesional chemotherapy with vinblastine sulfate (0.2–0.4 mg/ml per cm^2 of lesion) or sclerotherapy with 3% sodium tetradecyl sulfate (0.1–0.2 ml per cm^2 of lesion) is effective. Radiation therapy (800–2,000 cGy) is effective for larger or multiple lesions; stomatitis and glossitis are common side effect of radiation, but xerostomia is not.

Non-Hodgkin's Lymphoma (NHL). NHL is the most common lymphoma associated with HIV infection and is usually seen in late stages with CD4 lymphocyte counts of less than $100/mm^3$. It appears as a rapidly enlarging mass, less commonly as an ulcer or plaque, and most commonly on the palate or gingivae. NHL may be indistinguishable from masses caused by KS or other diseases in HIV-infected patients. Histological examination is essential for diagnosis and staging. Prognosis is poor, with mean survival time of less than one year, despite treatment with multi-drug chemotherapy (57).

Immune-Mediated Oral Lesions

While HIV infection and progression is characterized by progressive immune deterioration, it is equally well characterized by an abnormally activated immune system. In other words, the immune system activation itself leads to tissue injury and the worsening health of the patient.

Major aphthous ulceration is the most common immune-mediated HIV-related oral disorder, with a prevalence of approximately 2–3% (48, 49). The large solitary or multiple, chronic, deep, painful ulcerations of major aphthae appear identical to those in non-infected patients, but they often last much longer and are less responsive to therapy (Fig. 7). The diagnosis must include the possibility of a primarily infectious entity which can be determined by histological examination of biopsy material. Treatment requires the use of a potent topical steroid such as clobetesol (0.05% ointment applied for 45 seconds 3 times daily) when the lesion is accessible or dexamethasone oral rinse (0.5 mg/5 ml dexamethasone elixir 3 times daily, rinse for 45 seconds and expectorate)

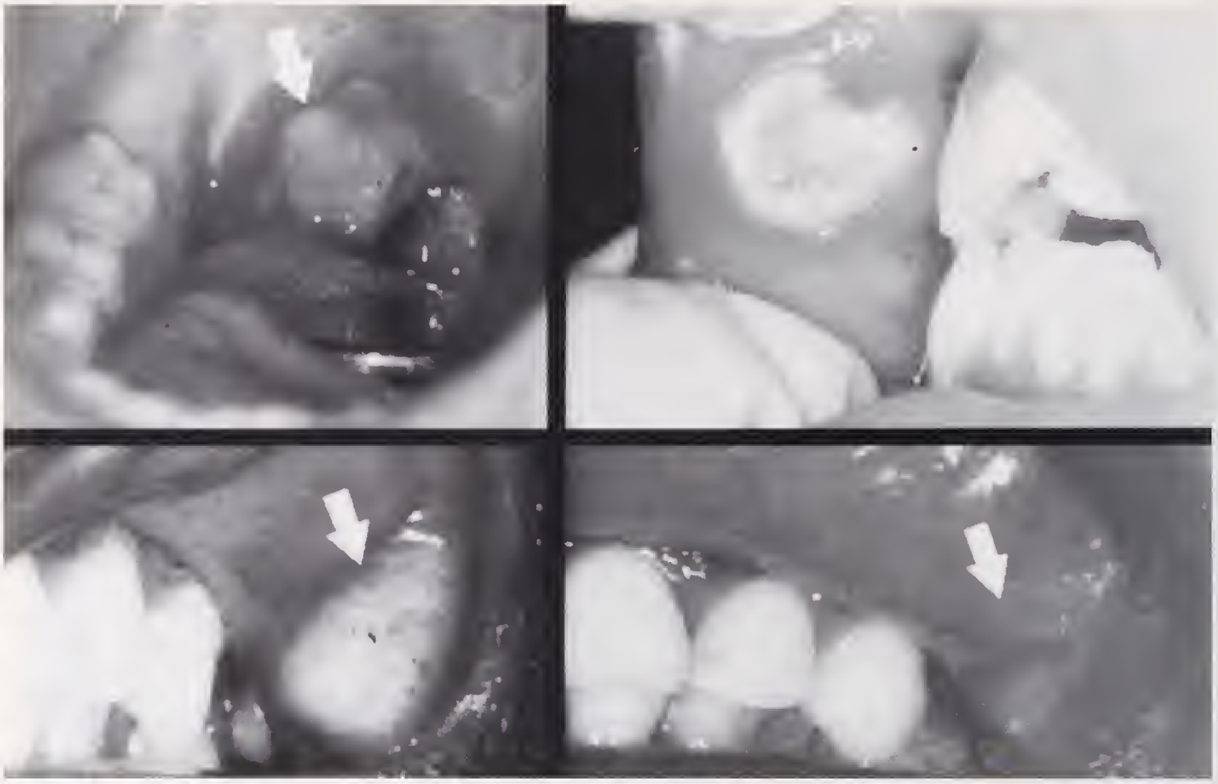

Fig. 7. Major aphthous ulceration of the palate (upper left) and buccal mucosa (upper right). Major aphthous ulceration of the lip before (lower left) and one week after (lower right) treatment with topical clobetesol ointment.

when inaccessible. When multiple ulcers are present or response to topical treatment is incomplete, systemic glucocorticosteroid therapy is required (prednisone 1 mg/kg). Since lesions often return after discontinuing medication, the use of prophylactic medication is not uncommon. Major aphthae are associated with advanced HIV infection (typically with CD4 lymphocyte counts of less than 50/mm^3). Long-term use of systemic prednisone may lead to complications such as oral candidiasis, reactivation of tuberculosis, or worsening of Kaposi's sarcoma. Alternative therapies such as dapsone 50–100 mg daily (58) and thalidomide 200 mg daily for 4 weeks (59) should be considered. When immunosuppressant or modulating drugs are used, concurrent antifungal medications such as fluconazole 100 mg tablet 1–2 daily, itraconazole 100 mg tablet 1–2 times daily, and antibacterial medications such as chlorhexidine gluconate oral rinse 0.12% 3 times daily, may be required to prevent superinfection or opportunistic overgrowth.

Necrotizing stomatitis is an uncommon acute, painful ulceration which often exposes underlying bone and leads to considerable tissue destruction. This lesion may be a variant of major aphthous ulceration, but occurs in areas overlying bone and is associated with severe immune deterioration. Unlike necrotizing ulcerative periodontitis, the lesion may occur in edentulous areas. As in major aphthous ulceration, systemic corticosteroid medication or topical steroid rinse is the treatment of choice.

Xerostomia

Xerostomia is common in HIV disease, most often as a side effect of antiviral medications or of the other antihypertensive, antidepressant, anxiolytic or analgesic medications commonly prescribed for patients with HIV infection. The oral dryness presents a significant risk factor for caries and can lead to rapid dental deterioration. Xerostomia also contributes to oral candidiasis, mucosal injury and dysphagia, and is often associated with pain and reduced oral intake of food. Although several saliva substitutes exist, compliance is often poor and relief inadequate. For patients with residual salivary gland function, determined by gustatory challenge, oral pilocarpine (5 mg up to 3 times daily) often provides improved salivary flow and consistency. Oral hygiene instruction, regular maintenance, and the use of prescription-strength, fluoridated dentifrice (Prevident 5000 Plus®) is essential.

Parotid Gland Disease

HIV infection is associated with parotid gland disease, characterized clinically by gland enlargement and diminished flow, and histologically by lymphoepithelial infiltration and benign cyst formation (50). The enlargement typically involves the tail of the parotid gland or, less commonly, the submandibular gland, and it may present uni- or bi-laterally with periods of increased or decreased size (Fig. 8). While the appearance may raise suspicion of malignancy (salivary gland or lymphoma) or infection, aspiration of a yellow mucinous secretion supports the diagnosis of HIV-related salivary gland disease, thus avoiding unnecessary biopsy or imaging diagnostics (51). Occasional swelling can be managed simply by repeated aspiration and rarely is radical removal of the gland necessary. The pathophysiological mechanism is not known, though cytomegalovirus has been suggested to play a role (52).

Pain Syndromes

Pain is a common symptom experienced by patients with HIV infection. Pain may result from a wide variety of disease processes, including direct effects of HIV on the central or peripheral nervous system, infection, malignancy, and antiretroviral therapy. Headache is a common symptom, occurring in approximately 46% of patients with HIV infection and accounting for approximately 17% of all pains in patients with HIV infection (53). Neuropathic pain is common among patients with HIV infection (19%), the most common diagnosis being painful peripheral sensory neuropathy. A complete discussion of pain syndromes in HIV infection is found elsewhere (53–55).

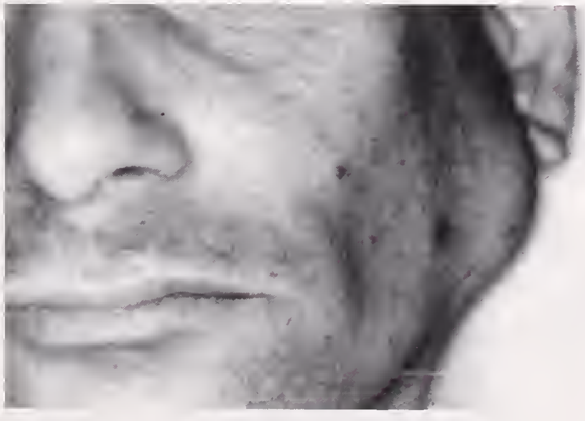

Fig. 8. HIV-related parotid gland disease. Gland can vary in size over time.

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Minor Oral Injuries in Children

Kathy Banks, D.M.D.[1], and Phyllis G. Merlino, D.D.S.[2]

Abstract

Traumatic injuries involving the oral cavity in children often result from falls or collisions with stationary objects. Repair of lacerations involving the soft tissue structures within the oral cavity is described. These injuries often can be managed by emergency department personnel, with referral to an oral and maxillofacial surgeon for follow-up care. Initial management of more extensive injuries such as dentoalveolar fractures, penetrating injuries and burns is also described. Immediate consultation with the appropriate specialist is suggested, because significant deformity and/or morbidity may result from minor oral injuries. **Key Words:** Oral soft tissue injuries, dentoalveolar trauma, pediatric patient.

Introduction

TRAUMATIC INJURIES INVOLVING THE ORAL CAVITY in children most often result from falls or collisions with stationary objects. Other incidents in which injury to the oral structures frequently occurs are sports-related accidents, motor vehicle accidents, and assault. According to one review of emergency department admissions of children, more than 50% of oral injuries were the result of falls. When the injuries were categorized, it was reported that lacerations to the lip account for 62% of the injuries, followed by injuries to the soft structures of the oral cavity. Injuries of the teeth and bones collectively accounted for only about 22% of the total injuries (1). Other studies (2, 3) report similar data. Accidental injuries such as electrical burns, penetrating injuries, and animal bites involving the oral cavity are also seen in children, but less often than lacerations and dental trauma. Most of these injuries require urgent or emergency treatment to prevent untoward sequelae. Given the myriad of situations with which emergency department personnel are presented, it is imperative that the attending physicians, dentists, and staff are well versed in the evaluation, treatment, and possible complications of major and minor oral injuries in children.

Maxillofacial injuries occur in about 20% of patients with multiple injuries (4). Life-threatening injuries to children, such as head and spinal cord injuries, thoracic and abdominal injuries, limb and craniomaxillofacial fractures, may be accompanied by traumatic injuries involving the oral structures. Such injuries necessitate immediate, comprehensive evaluation of the overall status of the child. Each child presenting for urgent or emergency treatment of injuries should be examined carefully for signs of abuse or neglect. Soft tissue injuries account for more than 90% of all injuries attributed to neglect or abuse (5). A description of the events leading up to and culminating in injury, and initial management of the injury by the parent or caretaker, including time lapse between injury and presentation for treatment, should be obtained from the patient and parent/caretaker, preferably in separate interviews. Discrepancies which may indicate patterns of abuse or neglect should be noted.

From the [1]Department of Oral and Maxillofacial Surgery, New Jersey Dental School, University of Medicine and Dentistry of New Jersey, Newark, NJ, and [2]Pediatric Dentistry Residency Program, Department of Dental and Oral Surgery, Brookdale Hospital University Medical Center, Brooklyn, NY. [1]Clinical Assistant Professor and Director of Anesthesia and [2]Resident.

Address correspondence to Kathy Banks, D.M.D., University of Medicine and Dentistry of New Jersey, New Jersey Dental School, Director of Anesthesia, Department of Oral and Maxillofacial Surgery, 110 Bergen Street, Newark, NJ 07103-2400.

Accounts of previous emergency room admissions should be obtained, including histories of the injuries for which treatment was sought. A seemingly excessive number of emergency room admissions may also point to a situation of abuse or neglect. Even in the majority of cases, where abuse or neglect is not suspected, accurate documentation of this information is required for medical and legal reasons. It is the obligation of all health care providers to report cases of suspected child abuse or neglect to the proper authorities.

In the otherwise stable patient, careful medical history, including vaccination history, should be obtained. The injured child should be observed for extraoral signs of injury, including swelling, changes in color of the skin, and asymmetry of the face and head. The skull, facial bones and mandible should be palpated to assess the mouth opening. Cranial nerve function should be thoroughly examined and evaluated. Any areas of ecchymosis, crepitus or pain upon palpation, or dysesthesia may indicate the presence of underlying fractures. The presence of emphysema in soft tissues of the head and neck is often indicative of fractures of the craniomaxillofacial skeleton. A thorough intraoral examination of the oral cavity and pharynx is performed, including inspection and palpation of the buccal and alveolar mucosa, teeth, alveolus, pharynx, tongue and floor of the mouth (6, 7). To rule out the possibility of aspiration, immediate chest and abdominal radiographs are required when teeth or portions of teeth are missing and unaccounted for. Soft tissue radiographs are helpful in determining displacement of radiopaque objects into adjacent soft tissues. Function of the major salivary glands is assessed by palpation of the gland while observing salivary flow from the respective duct orifices. Lacerations must be probed to evaluate the extent of the injury, and injury to adjacent structures.

Soft Tissue Injuries

Soft tissue injuries can be classified under categories according to the type of wound. The general types of soft tissue injuries that will be discussed are: contusion, abrasion, laceration, burns, and penetration injuries.

Contusions

A contusion (Fig. 1) results from injury to the soft tissues in which the epithelium or epidermis remains intact. Usually this type of injury is created when the soft tissues are crushed between two surfaces. For example, a contusion involving the soft tissues of the cheek results when they are crushed between the underlying bony prominence of the zygoma and a fist. The injured portion of the soft tissue is concentrated in the layers of the dermis and underlying muscles. It is possible for the deeper fascial layers to be involved. The crushing injures capillaries and blood vessels, initiating an inflammatory response in the tissues. Extravasated blood and escape of fluid from damaged cells cause the color change and swelling of the injured area. Since the protective barrier of the epithelium or epidermis remains intact, there is little risk of bacterial infection. Evaluation should include thorough examination for fractures of underlying bones. Severe pain, mobility, ecchymosis and air embolization in the soft tissues, which presents as crepitus upon palpation, are clinical indicators of fractures in the craniomaxillofacial area. Contusions should be treated with ice applied to the injured area to arrest further extravasation of blood by constricting capillaries. Warm compresses should be applied later to cause vasodilation and encourage lymphatic drainage. The extravasated blood will be broken down and removed, and revascularization of the area will result in complete healing (7).

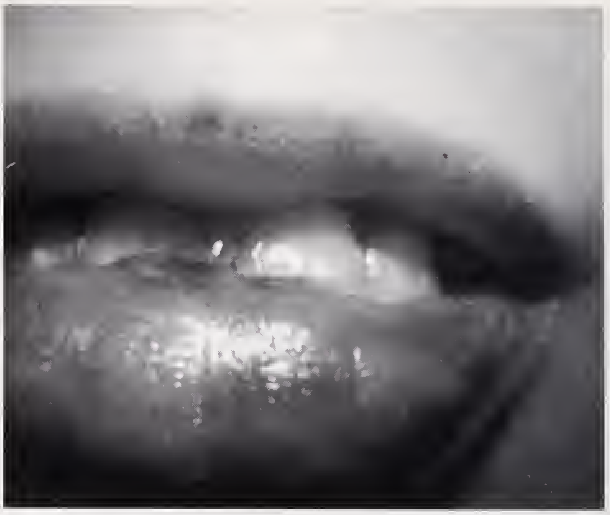

Fig. 1. Contusion injury to the lower lip illustrating edema and ecchymosis typically seen in this type of injury.

Abrasions

Abrasions are soft tissue injuries in which the epithelium or epidermis is removed, leaving the dermal elements exposed. "Brush burns" are abrasions that may follow a tangential blow which generates friction between the skin and a surface of the implement. Since the epithelium or epidermis is breached, abrasions are considered contaminated wounds. Treatment of abrasions includes initial cleansing of the injured area with an antibacterial solution followed by careful debridement of the wound to remove foreign material and devitalized tissue. Scrubbing the wound with brushes or sponges should be avoided since this practice may further injure and devitalize the dermal or submucosal tissues. Soft tissue radiographs may be helpful in determining if deeply embedded foreign materials remain in the wound following debridement. A final, thorough cleansing of the abrasion is followed by the application of a topical antibiotic cream. Due to the nature of the wounds, suturing is usually unnecessary. Systemic antibiotics are recommended in severely contaminated or deep abrasions, but should never be used in lieu of proper wound cleansing (7).

Immunization history should be obtained, since potentially dangerous pathogens such as *Clostridium tetani* may be introduced into the wound by dirt or gravel. Immunization against diphtheria, tetanus and pertussis is usually initiated within the first 6–8 weeks of life, with boosters at 18 months and when the child reaches school age. Thereafter, boosters are administered at 10-year intervals. At the time of injury, administration of tetanus toxoid is indicated to augment the protective antibody level only in the patient who has been previously immunized. Tetanus immune globulin and adsorbed tetanus toxoid are administered to the patient who has not previously been immunized against tetanus. The administration of tetanus antitoxin is indicated only if tetanus immune globulin is not available (8, 9).

Lacerations

Lacerations are wounds created by tearing of the soft tissues; these wounds may extend through all layers of the epidermis, dermis, and muscle layers, and into the fascial planes of the head and neck region. Perioral lacerations may extend from the skin surface, creating a communication with the oral cavity. Such lacerations are referred to as "through and through" lacerations. Lacerations are contaminated wounds, with the potential of bacterial inoculation from skin surface, or from the objects or surfaces causing the laceration. Evaluation of lacerations should include assessment of extent of the wound, including length, depth, affected structures, avulsion, and devitalization of tissue. Hemorrhage should be controlled immediately, with identification of the source of bleeding and ligation or cauterization of affected vessels. Lacerations should be thoroughly irrigated and debrided to remove foreign material, as well as devitalized and necrotic tissue, and to prepare the wound margins for closure. Lacerations involving the oral mucosa of the cheek mandate a careful examination of parotid salivary flow from Stenson's duct. The duct orifice is located on the buccal mucosa opposite the maxillary first molar. The flow of saliva from the orifice is observed while palpating the gland. Injuries involving these structures should be referred to an oral and maxillofacial surgeon for repair.

Simple closure of the oral mucosa with 3-0 chromic or plain gut suture is sufficient to promote adequate healing in minor lacerations. Deeper lacerations will require layered closure of the muscular layer followed by closure of the mucosa. 4-0 slow-resorbing polyglactin 910 suture is useful for the approximation of muscle, since it is more slowly resorbed and maintains wound strength for approximately 30 days. Antibiotic therapy is reserved for more severe wounds, grossly contaminated wounds, wounds with significant contusion of the marginal tissues, and wounds where there has been a delay in treatment. Penicillin, clindamycin, and erythromycin are appropriate antibiotics for empiric therapy against the oral flora.

Gingival lacerations occur when force applied to the lips creates tension on the gingival tissue such that the entire mucoperiosteal layer is traumatically elevated from the alveolar bone toward the apices of the teeth. This type of laceration usually involves one or more vertical tears, which may be partial to full in thickness. The alveolus should be observed directly for fractures or avulsions of alveolar bone and teeth, and the presence of foreign materials. Following thorough irrigation and removal of debris, reduction of alveolar bone and displaced teeth is accomplished. In severe degloving injuries involving the mucoperiosteal tissues, laceration may extend to the level of the muscle attachment. Deep, slow-resorbing sutures are used to repair the muscular layer. Failure to close lacerations within the muscle layer may result in soft tissue deformities that are difficult to correct in the postoperative phase. Closure of the gingiva is accomplished by replacing the displaced mucoperiosteal curtain and aligning the edges of the vertical components at the margin next to the teeth. It is best to avoid removal of any devitalized tissues, especially along the scalloped edge of the interdental papillae, until the mucoperiosteum has been properly repositioned.

Each of the interdental papillae will have a corresponding interdental embrasure into which it fits. Closure of the gingival lacerations begins with simple interrupted sutures passed through the central portion of the facial interdental papilla, through the embrasure space between the teeth, and through the palatal or lingual papilla. The suture is then passed back through the same embrasure space, without being passed through tissue, and tied on the facial aspect of the papilla. An absorbable suture such as 3-0 chromic gut with a 3/8 round cutting needle is used, since this needle is a good shape and size for suture passes through the interdental embrasures.

Lacerations of the frenum attachments will be V-shaped and include a small muscle layer deep in the mucosa. The muscular component of the frenum is responsible for hemorrhage that sometimes necessitates suturing. Otherwise, a small laceration involving the frenum may be irrigated and allowed to granulate secondarily (Fig. 2). When required, repair of small lacerations of the frenum is easily completed with one or two vertical midline absorbable sutures. Due to the nature of the muscular attachment of the frenum, some wounds may appear to be wide and gaping. Healing by secondary intention in these cases may lead to significant scar formation and interference with normal function and mobility of the tissues.

Tongue lacerations present a challenge to the surgeon, in that the tongue is a muscular organ with a large range of physical animation. It is also in constant danger of being injured by the teeth since it plays an essential role in the mastication and deglutition of the food bolus. Lacerations involving the tongue, especially those extending into the musculature, tend to gape open as the tongue is moved. The sutures placed need to withstand the trauma of the teeth and intrinsic muscle pull. Injuries to the tongue, especially those involving the ventral surface, are prone to hemorrhage, owing to the extensive collateral vascular blood supply. Significant edema of the tongue poses a very real risk of airway embarrassment via occlusion in the postoperative phase. Hospital admission is recommended for the patient with severe tongue injury and accompanying edema, in order to facilitate airway observation and maintenance. Tongue lacerations limited to the mucosal surface are closed with simple interrupted sutures. Deeper lacerations require layered closure, with the muscular layer and mucosal layers being closed separately. It may be advisable to bury the suture knots so that the patient is not prone to manipulate and break down the wound closure. Minor lacerations which are less then 1 cm in length may not require suturing, especially in the very young child, since the tongue has an enormous propensity for healing. Care must be exercised in suturing lacerations in the very young child, especially if the child is sedated, since blood and secretions pose an aspiration risk.

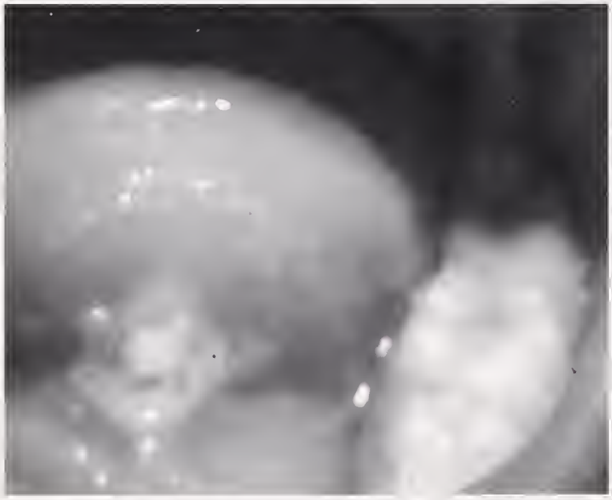

Fig. 2. Small laceration of the lingual frenum. The wound did not require surgical intervention and healed uneventfully by secondary intention.

A thorough understanding of lip anatomy is required for all lacerations involving the lips (9). The soft tissue contours of the philtral columns, the junction of the lip vermilion with the skin of the upper lip, and the cupid's bow form important aesthetic structures. Failure to align the edges of the vermilion and the skin properly will result in significant step deformity. The wet-dry line demarcates the transition from oral mucosa to lip vermilion. Alignment of the wet-dry line will also assist in the orientation of tissues. The orbicularis oris muscle will create tension on the wound when animated and must be properly repaired to restore proper function. Significant hemorrhage from the inferior or superior labial arteries may be brisk and require treatment (10).

Complicated lacerations crossing the vermilion border should be closed in a layered approach, beginning with repair of the orbicularis oris muscle. Closure of the oral mucosa is then completed with care taken to align the wet-dry line. Final closure of the skin and vermilion begins with a single suture carefully placed at the junction of the vermilion and skin wound margins to align the red line of the vermilion and the white line of the skin (10). Failure to properly align these structures will result in an esthetically unacceptable result. Wound care is performed twice daily with hydrogen peroxide followed by application of topical antibiotic ointment.

Electrical Burns Involving the Oral Cavity

Home safety precautions with respect to electrical appliances cannot be overemphasized, as shock, cardiac arrest, arrhythmias and even death may result from electrical burns. Oral electrical burns can produce devastating, full thickness destruction of the lip and cheek, resulting in severe functional disabilities and facial deformities (11, 12). Electrical burns occur when the child places the female end of a live extension cord in the mouth, or bites into a cord, resulting in injury to the commissure of the lip, tongue, or floor of the mouth. Electrical burns may also result from contact with the male end of an incompletely plugged-in cord (12–14) (Fig. 3).

The magnitude of the tissue injury depends on the duration of the contact, the voltage, tissue resistance, and path and type of current (14, 15). Visible electrical discharges or arcs do not require direct contact of the electrical source with the tissues to produce injury. Saliva serves as a conduction medium connecting the polarity gap of the wires of the electrical source. The electrical discharge in the form of an arc may produce temperatures exceeding 2500°C (12–15). Contact burns result from direct application of electricity to the tissues. In contact burns, the current is transmitted from the electrical source and travels through the tissues along the path of least resistance. The immediate response of the tissues to the applied energy is coagulation of the tissues, resulting in necrosis. At first presentation, there is a central gray or white zone of necrotic tissue surrounded by a raised, reddish, coagulated-appearing border. The adjacent tissues will be edematous. A fibrinous exudate will cover the wound in several hours. Coagulation of the tissues results in thrombosis of blood vessels and will usually prevent acute hemorrhage in the immediate post-injury phase. However, it should be kept in mind that bleeding from adjacent vascular structures, such as the labial arteries, might occur at any time following injury. The neural tissue component will also be damaged; thus, the injury may be surprisingly painless. Inflammation of the circumferential tissues progressively enlarges and peaks at about the first day following injury. As the wound matures, an eschar develops which will eventually slough, resulting in bleeding. There is a risk of significant hemorrhaging from arterial vessels during this phase. The resulting scar will have a tendency to contract, resulting in cosmetic and functional defects (11–15).

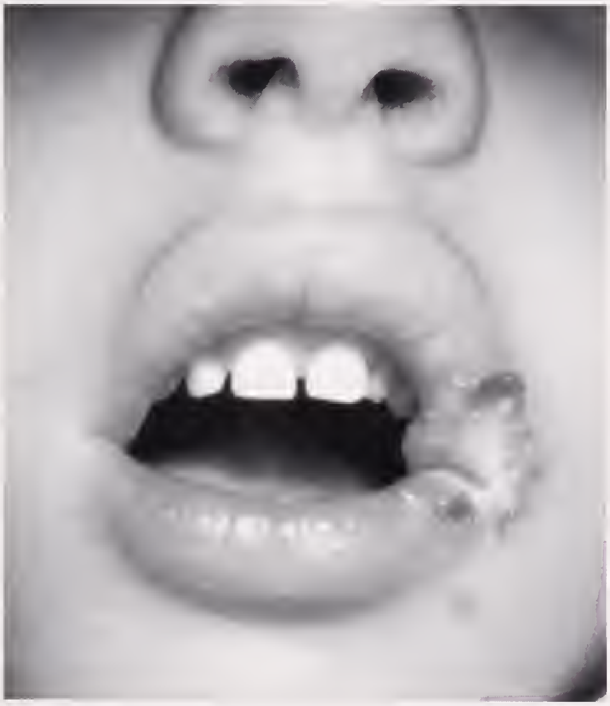

Fig. 3. Electrical burn involving the lip commissure, demonstrating the magnitude of tissue destruction and deformity of the oral structures. Note the central zone with peripheral erythematous margin demarcating the area of tissue necrosis, and edema of the adjacent soft tissues.

When the child presents following such an accident, initial management should include evaluation for systemic effects of electrical current such as shock and arrhythmias. If the child is otherwise stable, a thorough evaluation of the affected area is performed. Since the actual extent of tissue involvement cannot be determined in the immediate post-injury period (11, 12, 15), no attempt should be made to debride the wound. Electrical burns involving the tongue and floor of the mouth may result in damage to the vessels, culminating in uncontrollable bleeding, which requires ligation of vessels. The possibility of bleeding in the oral cavity places these patients at risk of aspiration. Edema of the tissues of the tongue surrounding the central necrotic zone and elevation of the tongue produced by swelling of the floor of the mouth place the patient in danger of airway occlusion. Swelling may also limit oral intake, placing the patient at risk of dehydration and nutritional deficits. Admission for airway observation and maintenance, as well as parenteral support, is justifiable and advisable in cases of severe electrical burn injuries of the orofacial region.

Considerable success has been reported with the use of appliances designed to stent the commissure during the healing and scar maturation phase (12–15). Appliances serve to maintain the dimension of the oral aperture and to prevent or limit the amount of scar contraction (12, 16). The appliances are usually designed in such a way that they are retained by the dentition in a removable or fixed manner stabilized by the dentition and alveolar ridges. Alternately, Barone et al. (16) describe an appliance that is retained by an earpiece resembling the temple of an eyeglass frame. The connection between the earpiece and the stent is elastic and allows for adjustment of the position of the commissure. Stenting the commissure during the healing phase may limit the magnitude of reconstructive surgery required in the future, or it may even eliminate the need for reconstruction altogether in certain cases (12, 16). Appliance therapy is usually initiated in the first weeks following injury, after the loss of the eschar. The appliance is worn for 6–12 months (12, 16). The need for surgical intervention and the timing of surgical repair or reconstruction are variable, depending on the needs of the patient and the practices of the treating surgeon.

Penetrating Injuries

A penetrating or puncture wound of the oral cavity is an accidental injury which most often results when a child falls with an object such as a pen or toy held in the mouth (Fig. 4). Occasionally, the child will be impaled when falling on an object with the mouth open (17–19). The resultant injury most often is a puncture or deep laceration located in the oral cavity. The most frequently involved sites are the posterior palatal areas and lateral pharyngeal walls (18). Severe hemorrhage is an unusual presentation; spontaneous cessation of insignificant hemorrhage is the usual case (18, 19). Evaluation of the patient includes taking the history of the injury followed by a thorough head and neck examination. The wound is first examined to determine the extent of the injury and the presence of foreign materials. Then the wound is observed as the patient performs maneuvers that animate the palatal vault and pharyngeal walls. The gag reflex is tested, and phonation is evaluated to determine the presence of air escape to the nasal cavity from the oral cavity.

Surgical intervention is indicated in cases of uncontrollable hemorrhage, severe laceration or avulsion of tissues, impaired function of the oropharyngeal walls, or in cases where it is suspected that foreign materials may be embedded in the wound. In most cases, however, the laceration or puncture wound does not require surgical management (17–19). If there is no significant gaping of the wound, no muscle contraction, and no foreign materials present, appropriate management includes thorough irrigation of the wound and observation during the healing phase. Possible complications include airway obstruction, symptomatic carotid artery injury, and loss of function (17–19). Localized infection may result from the introduction of bacteria present in the oral cavity or from the surface of the object entered into the tissues. Infrequent occurrences of facial cellulitis following oral penetrating injuries have been reported, but they are not common (17, 18). For this reason, appropriate prophylactic antibiotic therapy is advisable.

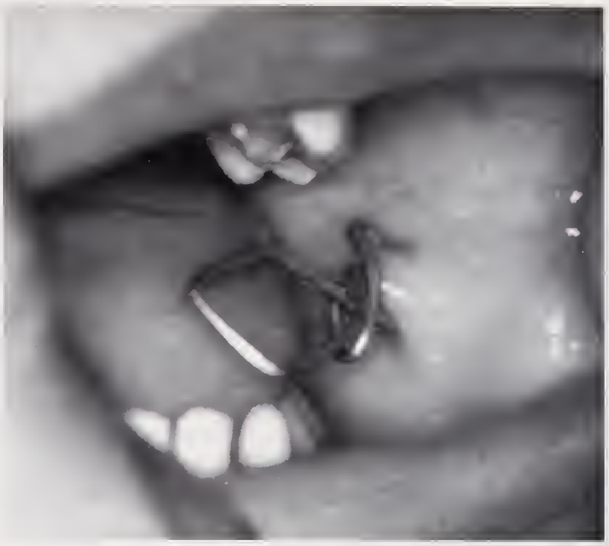

Fig. 4. Minor penetrating injury of the buccal mucosa, which requires only removal of the foreign object and observation during the healing phase. It is important to confirm protection against tetanus when penetration or puncture wounds are sustained.

Dentoalveolar Injuries

Dentoalveolar injuries include fracture, subluxation or avulsion of teeth, and fractures of the alveolar processes of the maxilla and mandible. The alveolar processes are those portions of the maxilla and the mandible which house and support the dentition. Dentoalveolar injuries commonly result from falls, sports-related injuries, motor vehicle accidents, and assaults (20). In one study, patients less than 15 years of age accounted for more than 75% of injuries to teeth treated in hospital emergency rooms (21). While most dental injuries result from accidents at home or school (1, 2), a considerable proportion of dental injuries are related to sports (20–23). It has been shown in several studies that most dental injuries are preventable if protective devices such as mouthguards are used while participating in sports (22, 24). However, injuries to the teeth as a result of accidents in the home or from falls are not always foreseeable events. Several studies have identified anatomical or morphological characteristics that may predispose certain children to traumatic injuries to the teeth. These include a forward position of the maxilla and procumbent maxillary incisors with respect to the position of the lower jaw and teeth, and inability of the upper and lower lips to meet when the teeth are interdigitated at the rest position; this is known as lip incompetence (25, 26).

Injuries to the teeth and dentoalveolar structures can potentially result in severe disfigurement. Disability as a result of injury or defects in the dentoalveolar apparatus may produce difficulty in phonation and masticatory function. Abnormalities in the appearance and function of the structures of the face and mouth have been shown to place patients at risk of developing problems with self-image and self-acceptance, and may lead to development of abnormal psychosocial behavior patterns (21). It is, therefore, important that every effort is made to preserve the integrity and function of the teeth and dentoalveolar structures.

Fractures of the teeth are rarely considered life-threatening emergency situations except in the case where there is aspiration of a tooth. If the tooth is not accounted for in the clinical examination, a chest radiograph is indicated to rule out this possibility. Prompt referral to a pulmonologist is required for retrieval in the case of aspiration. Swallowing of teeth is also a possibility, but carries a significantly lower morbidity than aspiration. The swallowed foreign object may be visualized on abdominal radiograph, and intervention is seldom required. Serial radiographs will track the tooth as it progresses through the gastrointestinal tract.

Even though it is possible for primary teeth to be fractured, intrusion, luxation or avulsion injuries are more common (27). The root and alveolar bone support of the primary teeth are minimal and may predispose the primary dentition to avulsion and luxation injuries. The permanent teeth are more firmly embedded in alveolar bone and may be more likely to fracture.

Fractures of the teeth limited to the enamel do not generally produce pain, but the tooth may feel rough and irritate the tongue, lip or mucosa. If exposure of the tooth to air or cold fluids causes mild to moderate sensitivity, these stimuli should be avoided. The patient should be referred to a dentist for follow-up care and treatment (6, 24). Fractures involving the enamel and dentin may be exquisitely sensitive, especially when exposed to cold air or liquids. Unless there is a contraindication, pain can be alleviated temporarily by infiltration of 1 – 2 ml of local anesthetic solution, such as 1% lidocaine, into the buccal gingival mucosa over the root apices of the injured tooth. This technique will reliably produce pulpal anesthesia in most teeth. Mandibular molars may require a dental nerve block of the inferior alveolar nerve to alleviate pain. In almost all cases, the injured tooth is restored with a good prognosis (6, 24). Fractures of the crown of the tooth, in which the dental pulpal tissues are exposed, may also be painful. The administration of local anesthesia will alleviate pain temporarily and allow for careful examination. The direct exposure of the pulpal tissues to the oral cavity provides an entry route for bacteria. Infection of the pulpal tissues will ensue if treatment is delayed beyond several days. Most often, the tooth will require endodontic therapy. Prompt follow up with a dentist is recommended (6, 24).

Vertical fractures of teeth may be painful as a result of movement of loose portions that will stimulate pain fibers in the periodontal ligament system. On examination, bleeding from the sulcus of the tooth is usually minimal. This bleeding stops spontaneously and requires no intervention. If the fractured portion of the tooth is quite mobile, it may become dislodged. In this case, the tooth should be removed to prevent possible aspiration. Infiltration of local anesthetic is administered to relieve pain and allow for removal of the fragment. The airway should be protected with a gauze screen and the tooth fragment grasped with a small rongeur or hemostat for removal. The prognosis of restoring the tooth depends on the extent of damage to the tooth. In general, the further the vertical fracture extends toward the apex of the root, the poorer the prognosis.

Horizontal fractures involving the roots of teeth are suspected when there is mobility of a tooth following a blow to the mouth or teeth. The tooth may be displaced and there may be bleeding from the gingival collar of the tooth. These injuries may cause pain due to stimuli within the periodontal ligament structure and the dental pulp. Dental radiographs may be used to confirm suspicion of root fracture. Interim care includes relief of pain and stabilization of the tooth if it is very mobile (6). There are a variety of methods used to stabilize the tooth. As described by Medford (28), periodontal dressing material may be placed to provide short-term, temporary stabilization. Other, more time-consuming and technically difficult means of stabilizing the tooth include the application of a wire splint to the teeth with dental bonding material and the use of an arch bar with circumdental wiring. The prognosis of the tooth is determined by the position of the fracture with respect to the apex of the tooth. In general, the closer the fracture is to the apex of the tooth, the better the prognosis. Definitive treatment usually requires endodontic therapy and possibly extraction of the unsalvageable tooth.

Fully formed permanent teeth which are subluxated (displaced, but still positioned within the socket) have an excellent prognosis if properly managed. Subluxation injuries involving teeth with incompletely formed root structures have a poorer prognosis, because the dentinal formation within the root structure will be compromised and more susceptible to subsequent root fracture (29). On examination, the tooth will be displaced but still positioned within the socket. A more severe type of luxation injury is the intruded tooth, where the tooth is traumatically displaced deeper within the alveolar bone. The presence of associated gingival lacerations may be indicative of involvement of the alveolar bone. There will be significant pain due to disruption of the periodontal ligament and injury to the gingival tissues. Infiltration of local anesthesia to the buccal mucosa and gingival tissue will provide relief from pain and allow the physician to temporarily stabilize the tooth. Following thorough irrigation with a physiologic solution, the tooth should be replaced in the proper position in the socket with bimanual palpation. The patient should then close gently on a gauze sponge to assure complete apical seating. The tooth can be stabilized by any of the methods described above.

Displacement of primary teeth is common in children following trauma to the orofacial region; the child may complain of pain and the tooth may be displaced within the socket either laterally, medially, or vertically. There is usually bleeding from the gingival collar around the injured tooth, and there may be associated gingival lacerations. The injured area should be thoroughly irrigated with physiologic solution, followed by debridement of foreign materials. Infiltration of 0.5 – 1 ml of local anesthetic with vasoconstrictor into the gingival tissues surrounding the tooth will usually be adequate to relieve pain and allow the repositioning of the tooth through bimanual manipulation. Intruded primary teeth may erupt spontaneously and can be left in the intruded position. If the tooth is very loose following reduction, it presents a possible risk of aspiration and should be removed. Teeth which are not significantly loose do not require stabilization. In most instances, the displaced primary teeth will have a rather poor prognosis.

The prognosis of an avulsed permanent tooth depends on several factors: the amount of time spent out of the mouth, the storage method used to transport the tooth, the damage to the tooth, the condition of the periodontal ligaments still attached to the tooth, whether the tooth can be replaced and adequately stabilized (6, 24). The exposure of the tooth to air will desiccate the periodontal ligament fibers and the cementum layer of the tooth. Every effort should be made to maintain the tooth in a moist environment. The tooth may be transported in the buccal vestibule of the mouth, in milk, or in any of the commercially available tooth transport systems. If the emergency room admission is preceded by a telephone call, the parent should be instructed to rinse the tooth under running water and then to place the tooth into the socket immediately. The best prognosis occurs when the tooth is replanted within the first twenty minutes (6, 24). At the very least, teeth should be replanted upon arrival at the emergency room. Ideally, a wire splint fixated to the tooth with dental bonding material is used to stabilize it. Periodontal dressing material can be used to provide temporary stabilization of the tooth until the patient can seek care from a dentist. Primary teeth should never be replanted, as the developing permanent tooth may be injured.

When excessive traumatic forces are applied to the teeth and mouth, the resultant injuries may involve not only the teeth but also the alveolar bone supporting the teeth. Signs of dentoalveolar fractures include displacement and mobility of a portion of the alveolar process containing one or more teeth. There is a noticeable discrepancy in the level and alignment of the teeth when compared to the other teeth in the dental arch (Fig. 5). In children, occlusal dental radiographs and panoramic images are useful in confirming the presence of suspected fracture. Dentoalveolar fractures are open, contaminated fractures. There is significant risk of infection due to the plethora of bacteria that normally inhabit the mouth. Additionally, there is the possibility of bacteria being introduced via foreign materials embedded within the tissues. Empiric antibiotic therapy should be initiated prophylactically. Penicillin is a good choice due to its activity against the oral flora, but erythromycin or clindamycin may be used in the penicillin-allergic patient. Every attempt should be made to ensure the best prognosis for the fractured alveolar bone and involved teeth. Improperly treated injuries may result in

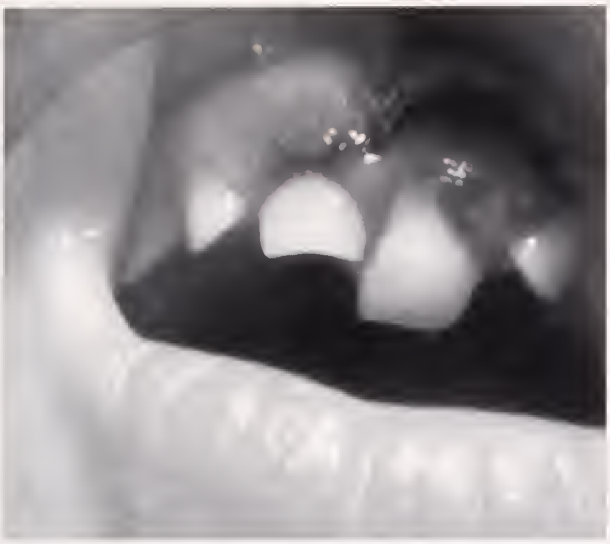

Fig. 5. Dentoalveolar injury involving anterior teeth, demonstrating the malposition of the teeth with respect to the alignment of the dental arch. Note the gingival laceration and the bleeding of the gingival sulcus, which are indicative of dentoalveolar trauma.

malalignment of the teeth and alveolar bone or in the loss of the entire dentoalveolar segment. If the teeth are improperly aligned or the fracture is not adequately reduced, major functional interference may occur due to tooth position. The loss of teeth coupled with the loss of the alveolar bone results in significant functional and cosmetic deformity. Prosthetic rehabilitation of the missing tissues is most often an unsatisfactory substitution for the missing teeth and alveolar bone. Extensive surgical reconstruction, including bone and tissue grafting to the deficient area, followed by prosthetic restoration with dental implants, is the best alternative when replacing the dentoalveolar components. Unfortunately, these procedures are not always successful and the cost may be prohibitive for many patients.

Conclusion

Management of traumatic and accidental injuries in children is often complicated by the emotional response of the patient and parent. The emergency department setting provides an environment that is not conducive to cooperative patient behavior in most children. As some injuries are extensive and have the potential for creating functional, esthetic, and psychosocial problems in the developing child, it is important to carefully evaluate each step of the treatment performed. Every effort must be made to preserve the permanent dentition, supporting dentoalveolar structures, and perioral soft tissues. Even minor injuries can cause permanent damage when treated improperly. When the behavior of the patient interferes with the correct treatment of the injury, employment of alternate management techniques such as sedation, or even general anesthesia, should be considered. Deformities resulting from the improper treatment of injuries to the pediatric patient are avoidable when proper techniques are used and timely referral is made.

Acknowledgments

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Burning Mouth Syndrome

Steven A. Miyamoto, D.D.S.[1], and Vincent B. Ziccardi, M.D., D.D.S.[2]

Abstract

Complaint of a burning mouth is an increasingly common problem in the aging population. This has remained an enigma for the treating clinician, because visible pathologic lesions or processes are usually not evident. Local, systemic and environmental causes must be assessed to elicit the predisposing factors. Some suggestions for managing burning mouth syndrome are offered. **Key Words:** Burning mouth, glossodynia, xerostomia, dysgeusia, dysphagia.

Introduction

Burning mouth syndrome (BMS) is a burning or stinging of the mucosa, lips, and/or tongue, in the absence of visible mucosal lesions. Van der Waal (1) defined the term "burning mouth syndrome" to refer only to idiopathic cases in which the main symptoms are located in the oral mucosa, with or without involvement of the tongue or any other part of the body. It has been estimated to affect more than one million Americans (2). There is a strong female predilection, with most female patients being postmenopausal and the age of onset being approximately 50 years (3, 4). The causes of BMS are multifactorial and remain poorly understood. Patients suffering from BMS are often evaluated by several clinicians, including the general practitioner, internist, otolaryngologists, dentists, oral and maxillofacial surgeons, oral medicine practitioners, and pain specialists. For this reason, it is important that a wide variety of specialists be able to recognize the signs and various causes of burning mouth syndrome, particularly those which fall outside of their specialties.

Symptoms

There are many symptoms associated with BMS which generally do not conform to anatomic boundaries. The tip of the tongue is the most common location (71%), followed by the lips (50%), lateral borders of the tongue (46%), dorsum of the tongue (46%), and palate (46%) (5). Lamb et al. (6) developed a classification system to group the varied course of symptoms. BMS type 1 is defined as the absence of symptoms upon awakening, with gradual increase in severity as the day progresses. Type 2 patients describe the burning as being present day and night. Type 3 patients are characterized as those with days of remission which follows no specific pattern.

A number of associated oral complaints have been observed concomitantly with the onset of BMS. Xerostomia, dryness of the mouth, is the most commonly reported symptom, occurring in approximately 50% of patients (1). In a study of salivary flow rates comparing BMS patients and asymptomatic patients, a decrease in salivary flow was not found to be associated with BMS (7). However, the salivary total protein and IgA concentrations were higher in BMS patients (8). Other commonly associated oral complaints include alteration in taste (dysgeusia), constant

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From the Department of Oral and Maxillofacial Surgery, New Jersey Dental School, University of Medicine and Dentistry of New Jersey, Newark, NJ. [1]Chief Resident, and [2]Assistant Professor and Residency Program Director.

Address correspondence to Dr. Vincent B. Ziccardi, M.D., D.D.S., University of Medicine and Dentistry of New Jersey, New Jersey Dental School, Department of Oral and Maxillofacial Surgery, 110 Bergen Street, Room B 854, Newark, NJ 07103-2400.

thirst, difficulty swallowing (dysphagia), and symptoms from the temporomandibular joint (9, 10).

Local Causes of Burning Mouth Syndrome

Although many predisposing factors have been attributed to BMS, all are controversial. Main and Basker (11) found ill-fitting dentures to be the single greatest contributor to BMS in their patient population. BMS patients were found to have significantly less daily denture use, reduced tongue space, incorrect placement of the denture occlusal table, and increased denture vertical dimension (12). However, in the majority of patients in whom denture abnormalities were adequately corrected, the burning mouth symptoms persisted (13).

A similar controversy attributes the cause of burning mouth to an allergic response to the denture materials. Methyl-methacrylate monomer and other products used in denture fabrication have been shown to produce positive skin reactions to patch testing (14). If erythema of the mucosa exists, the construction of new dentures using different materials, such as metal alloys, has been shown to result in a partial or even total relief of symptoms. However, in the absence of oral lesions which could indicate contact dermatitis, the substitution of materials in general has not been shown to alleviate BMS (15).

Hypersensitivity to mercury was found to be one of the most common medical diagnoses concomitant with BMS (10). In addition to mercury, other metals used in dental restorations are gold, palladium, zinc, tin, gallium, indium, cobalt, chrome, nickel, iron, and silicon. Although hypersensitivity to these materials has been reported, their precise role in the symptoms of BMS is not absolutely clear.

The symptoms of galvanism may resemble those of BMS, and they require consideration (1). Galvanism is the electrochemical reaction between different metallic restorations, such as dental amalgam and cast gold, in the presence of a conducting medium, i.e., saliva. The production of electrical currents can produce a sharp or burning sensation with or without an associated metallic taste. Treatment would involve the elimination of one of the metallic restorations or using synthetic nonconductive materials wherever applicable. As with BMS, there is a female predilection.

Pseudomembranous and erythematous candidiasis have been associated with BMS (16). The pseudomembranous type is easily recognized by white, slightly elevated plaques that have a milky appearance and can be rubbed off; it is most commonly found on the cheeks and palate. The erythematous type is characterized by flat, red changes of the mucosa, tongue, and palate. The diagnosis of candidiasis is often presumptive, made on the response to antifungal therapy and rarely from cytologic or histologic studies. Gorsky et al. (17) reported that in patients with BMS with no clinical signs of candidiasis, 86% improved after using antifungal lozenges and 13% had complete elimination of their symptoms.

Carcinomas of the oral cavity can also present with itching or burning as the first symptom (1). Oral carcinoma usually presents as an indurated, painful, ulcerated mass that is adherent to the underlying tissues. The most common sites for oral carcinoma include the tongue, floor of the mouth, mucosa of the alveolar ridges, buccal mucosa, and lower lip. Premalignant entities such as leukoplakia (a white adherent patch) or erythroplakia (one with areas of red intermingled within) may also present with burning or painful sensation. A biopsy should always be used to make the diagnosis and appropriate referral.

Systemic Causes of Burning Mouth Syndrome

Various systemic factors have been associated with BMS, although many of these conditions require further study to verify the correlation. There is a predilection for BMS to occur in menopausal and postmenopausal women (8); however, there have been varied opinions regarding the hormonal role in BMS. Oral discomfort, including burning mouth, is one of the two most common oral manifestations of menopause (18). Basker et al. (19) reported that 26% of patients evaluated for menopause experienced some oral symptoms, one-third of these describing BMS. Yet in early studies, neither topical nor systemic hormonal replacement therapy has been shown to be effective (20, 21). In a recent study, hormone-replacement therapy was found to be efficacious in BMS patients who had demonstrated nuclear estrogen receptors on immunohistochemical assay, and ineffective in those patients who did not have the receptors (22). Therefore, hormone modifications may have a role in selected patients with BMS.

Iron deficiency anemia and vitamin B complex deficiency have been considered possible factors associated with BMS (10, 23, 24). In one study (25), replacement therapy of vitamin B1, B2, and B6 was effective in treating BMS in 88% of patients. However, in another study of therapy-resistant BMS patients, vitamin B replacement therapy was unsuccessful (26). The definitive role of B complex vitamins in the etiology of BMS remains unclear.

Diabetes mellitus has been linked to BMS (19, 27) with an incidence rate of 10% (1) to 37% (27). It has been suggested that diabetics are more susceptible to the *Candida* infections which may cause the burning mouth (16). In addition, vascular changes that characteristically occur in diabetes cause changes in the small blood vessels of the mouth and thereby lower pain thresholds. While some found that the symptoms of BMS in diabetic patients did not decrease after glucose control (1), others found that in many cases, diabetic treatment resolved the oral symptoms (27).

Since burning mouth syndrome often has no clear etiology, an underlying psychiatric cause is often investigated. In one study, a psychiatric diagnosis was present in more than 50% of BMS patients, with depression as the most common disorder (28). In another, anxiety was reported to be more common than depression as a feature of the syndrome (29). BMS may be triggered by the significantly altered salivary composition caused by sympathetic activation resulting from psychological stress (8). However, while BMS patients may have elevated psychological stress, the onset of BMS symptoms has not been found to be related to stressful life events (30). Van der Waal (1) believes that it is far more likely that chronic intense symptoms of burning mouth may affect a patient's psychologic status rather than the reverse. Although stress and anxiety may exacerbate the problem, it has not been proven to be the definitive cause.

Diagnostic Work-up for Burning Mouth Syndrome

Since localized burning or itching sensation can be the first indication of the presence of a malignant lesion, a careful and thorough physical examination of the oral cavity should be performed. Random biopsies of normal-appearing oral mucosa and histologic examination of the buccal mucosa in 24 burning mouth patients did not show any abnormality (1). Underlying systemic diseases such as iron deficiency and pernicious anemia can be ruled out with appropriate blood work. Laboratory tests that should be considered include CBC, MCV, MCH, WBC, vitamin B12 (with B1, B2 and B6), folate, serum iron, TIBC, ferritin levels, and (%) transferrin saturation (1, 16).

Some medications have been implicated as possible causes of BMS (24). Drug substitution or lowering the dose of a drug to verify its role in BMS may be considered after discussion with the internist (1). In particular, antihypertensives such as ACE inhibitors have recently been implicated in some BMS cases (31).

Treatment of Burning Mouth Syndrome

If an underlying cause of BMS can be identified, treatment should be directed toward the source. If indicated, nutritional or estrogen replacement therapy should be initiated (32).

The scientific literature is replete with various palliative remedies which range from the common to the exotic. Fungicides, such as nystatin, have been shown to be effective and may be prescribed for anywhere from weeks to months, even when cultures fail to identify the causative organism (17, 32). Another remedy which has been described is a local application at the burning sites of a solution containing 7% salicylic acid in 70% alcohol soaked cotton pellets for about 10 seconds (1). Topical capsaicin has been reported to be beneficial in a few patients (32). Other concoctions, such as the "magic swizzle" (one part Kaopectate®, one part diphenhydramine, and one part viscous lidocaine), have been used with some success. A recent article by Regnard et al. (33) advocated topical anesthesia such as benzydamine mouthwash, choline salicylate, mucaine, or lozenges containing local anesthetics. Van der Waal (1) reports that the previously advocated use of a viscous solution of 2% lidocaine hydrochloride in a carboxymethylcellulose sodium and water vehicle has not been effective for therapy, since the analgesic effect has a short duration.

Antidepressants have also been suggested as treatment for BMS when other treatments have failed, or if depression accompanies the pain (2, 32, 34). The mechanism of action by which tricyclic antidepressants work to provide analgesia is unclear. However, those medications with both serotoninergic and noradrenergic effects, such as amitriptyline or doxepin, appear to be the most effective clinically. Amitriptyline should be started initially at a low dose and titrated to affect, beginning at 25 mg, or even 10 mg, in the elderly, daily or at bedtime (35). The analgesic effect usually appears at these low doses before they have effect on mood elevation (36), and is evident at one week for most patients. Increases of 25 mg can be made at weekly intervals if the analgesic effect is inadequate and the adverse effects are not pronounced. The most common adverse effects included drowsiness and xerostomia, but neither was found to be significant at these low doses. It is not known if doses greater than 75 mg produce more analgesia without significant adverse effects (35).

Even among patients whose symptoms persist lifelong, the majority adjust to the symptoms and give up seeking treatment (1), particularly if the clinician demonstrates a supportive and caring attitude (37). Patients with BMS refractory to other treatment should undergo psychiatric investigation (38). A psychological approach therapy has been used successfully to treat burning mouth syndrome (39). Some believe that psychotherapy combined with antidepressants is the most effective treatment when other causative factors are eliminated (4, 40).

Clearly, there are many opinions regarding the cause, proper diagnosis, and treatment of burning mouth syndrome. However, further research on BMS is required, as at this time there are no well-documented long-term studies of large numbers of BMS patients. In the final analysis, the clinician must recognize that burning mouth syndrome is a multifactorial disease process in which numerous possible etiologies must be eliminated before the proper treatment can be initiated.

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Differential Diagnosis of Orofacial Pain

Richard A. Pertes, D.D.S.

Abstract

Orofacial pain, especially if the problem is chronic, presents a diagnostic and management challenge to all health practitioners. This paper suggests how clinicians might simplify the diagnosis of orofacial pain. First, the pain is classified into one of the three basic pain categories: somatic, neuropathic, or psychogenic pain. Somatic pain results from noxious stimulation of normal neural structures. Neuropathic pain is caused by a structural abnormality in the nervous system. Psychogenic pain arises from psychic causes; there is no apparent physiologic or organic basis for the pain. The next step is to determine the tissue system from which the pain arises: intracranial, extracranial, musculoskeletal, neurovascular, neurogenous, or psychological. Finally, some of the more common orofacial pain syndromes within each category are discussed. **Key Words:** Somatic orofacial pain, neuropathic orofacial pain.

Introduction

Diagnosing orofacial pain can be quite difficult and frequently presents a formidable challenge to even the most astute clinician. Patients may have multiple pain complaints and often present with a confusing constellation of signs and symptoms, which can result in a variety of diagnostic possibilities. For example, does the patient who presents with facial pain after chewing have a temporomandibular disorder (TMD), or could symptoms be related to a neuropathic disorder that was triggered by the chewing? This paper will offer a road map in an attempt to simplify diagnosis of some of the more common orofacial pain syndromes.

The Diagnostic Process

The most important part of the diagnostic process is the history, which should include the following information concerning pain symptoms: (1) chief complaint(s); (2) characteristics of the pain(s) (location, mode of onset, quality, intensity, temporal behavior); (3) history of the present illness including results of previous examinations and treatment, and current medications; (4) any associated symptoms; (5) factors precipitating, aggravating, or relieving the pain; and (6) personal and past medical history. A physical examination should supplement the history. Various diagnostic aids such as imaging studies, laboratory tests, diagnostic anesthetic injections, and consultations with other health care practitioners may also be employed. In some cases, trial therapy may be necessary to confirm the diagnosis (1, 2).

Pain Categories

Using the information obtained from the history, physical examination, and diagnostic tests, the clinician should determine which pain category (somatic, neuropathic, or psychogenic) best describes the patient's chief complaints.

Somatic Pain

Somatic pain results from the noxious stimulation of normal structures that innervate the

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From the Division of Oral Medicine, Department of Oral Pathology, Oral Biology, and Diagnostic Sciences, New Jersey Dental School, University of Medicine and Dentistry of New Jersey, Newark, NJ.

Address correspondence to Richard A. Pertes, D.D.S., University of Medicine and Dentistry of New Jersey, New Jersey Dental School, Division of Oral Medicine, 110 Bergen Street, Newark, NJ 07103-2400.

affected area and is divided into superficial or deep somatic pain. Superficial somatic pain has a bright and stimulating quality, is primarily involved in acute pain conditions (e.g., mucogingival lesion) and usually does not present any diagnostic difficulty. In contrast, deep somatic pain arises from deeper body structures, has a dull, deep, depressing quality, and frequently exhibits CNS secondary excitatory effects such as referred pain to other sites and local autonomic effects (e.g., lacrimation, local edema, tearing of the eyes, and skin flushing). As a result, diagnosis can be elusive (1–3).

Neuropathic Pain

Neuropathic pain is due to a structural abnormality in one or more components of the nervous system that innervate the affected area. It can arise spontaneously in the absence of any obvious noxious stimulation and is usually described as bright, stimulating, and burning. Neuropathic pain may be accompanied by paresthesia along a distinct nerve distribution, and the severity of the pain is often out of proportion to the degree of stimulation; even light touch can cause intense pain (1, 3).

Psychogenic Pain

Psychogenic pain originates in the mind; it is not elicited by noxious stimuli or any abnormality within the neural system. Although the patient has physical symptoms, there is no apparent physiologic or organic basis for the pain. Psychological factors are involved in the etiology, and a definite history of emotional or personality disorder is present (2, 4). Because an anatomic relationship between the pain source and the site of pain is absent, psychogenic pain may be felt in many areas, the location varying during the disease process. The degree of pain expressed by the patient is often exaggerated, and response to treatment is usually inconsistent (3–5).

Diagnostic Groups

After establishing the pain category, the next step in the diagnostic process is to determine from which diagnostic group the pain originates. The clinician should then be able to identify specific pain syndromes on the basis of clinical characteristics as follows: (1) intracranial, (2) extracranial, (3) musculoskeletal, (4) neurovascular, (5) neurological, and (6) psychological (6).

Intracranial Structures

Although pathology of intracranial structures is an uncommon cause of orofacial pain, it must be considered because of its serious consequences. Pain arising from a space-occupying lesion (e.g., tumor, edema) has a deep, dull, steady, aching quality, is usually progressive, and often has associated neurological signs or deficits such as weakness, sensory loss, or dizziness. Red flags for an intracranial lesion include a new onset headache in any patient older than 40 years of age or a change in the nature of a chronic headache, and pain accompanied by neurologic signs or deficits (7, 8).

Extracranial Structures

Extracranial disorders include some of the more common disorders affecting the specialized structures: teeth, ears, eyes, nose, throat, sinuses, tongue, and glands. Because dental pathology is the most frequent cause of pain in and around the face and mouth, a good rule of thumb is to eliminate the teeth as the primary source of pain before proceeding to other suspected tissue systems. Odontogenic pain is of the deep somatic type and may exhibit a variety of central excitatory effects including referred pain, autonomic effects, and the induction of co-contraction and trigger points in muscles innervated by the trigeminal nerve (1, 9).

Orofacial pain is frequently due to maxillary sinusitis, located in the maxillary posterior teeth and face on the affected side. The mild-aching, non-pulsating pain is often associated with a feeling of fullness over the involved sinus, ear pain, deep eye pain, frontal headache, malaise, fever, nasal congestion, and nasal discharge. Pain does not originate from the lining of the maxillary sinus, as is commonly thought, but rather from the pain-sensitive structure of the nasal mucosa. Lowering of the head often increases pain by allowing any inflammatory exudate in the sinus to spread to the nasal mucosa, thereby causing referred pain to maxillary teeth. Opacification of the affected sinus may be detected by transillumination. Positive radiographic findings of inflammation in the sinus, along with elimination of pain by spraying local anesthetic into the nostril, confirm the diagnosis of maxillary sinusitis. Chronic sinusitis, as opposed to acute sinusitis, rarely causes facial or tooth pain (1, 10–13).

Musculoskeletal Pain

Musculoskeletal pain falls into the category of deep somatic pain and is described as constant, dull, and aching, with occasional exacerbations of sharp pain. Functional activity, manual palpation of the affected area, and tests of provocation all increase the pain. This diagnostic group includes temporomandibular disorders (TMD), fibromyalgia, disorders of the cervical spine, and the various rheumatic and collagen diseases that can affect the muscles and joints involved in chewing and the cervical spine.

TMD consist of a group of related conditions which all impact adversely on mastication. Common patient complaints include pain in the preauricular area, jaw, face, temples, and ear, as well as limited mandibular movement and joint sounds during function. Cervical pain and dysfunction frequently accompany TMD (14).

Pains originating in the skeletal muscle, its tendinous attachment, and investing fascia are the most frequent causes of discomfort in the head and neck region, and can even refer pain to the teeth. This pain is usually diffuse and characterized by variability ranging from a feeling of pressure to excruciating intensity. Muscle pain can be divided into acute and chronic muscle disorders (15). Acute muscle disorders include muscle spasm, which is a sudden, involuntary contraction of a muscle or group of muscles, accompanied by pain, limited vertical opening of the mandible, and tenderness to palpation. Another acute muscle disorder is myositis, an inflammation of the muscle due to a local cause such as trauma, strain, or infection. Symptoms include swelling, tenderness to palpation, and pain with function (15).

The most common chronic muscular pain disorder is myofascial pain, a regional aching pain associated with localized tenderness in firm bands of muscle and tendons called trigger points (TrPs). Myofascial TrPs can develop in masticatory and associated head and neck muscles secondary to prolonged muscle tension, protracted muscle spasm, forward head posture, parafunctional activity, and trauma. Factors such as sleep disturbances, joint problems, viral diseases, and metabolic disturbances that weaken a muscle often predispose a weakened muscle to the development of TrPs (15 – 17). The key to diagnosing myofascial pain is to identify a TrP. When palpated, active TrPs cause referred pain in predictable patterns to a known reference zone and are often associated with secondary excitatory effects. Continuous, dull pain and localized tenderness in one or more muscles are characteristics of myofascial pain, but the severity of pain may range from mild to agonizing, excruciating, or incapacitating. In early stages, the pain may be more localized, but with chronicity, the TrP can refer pain to more distant sites (15 – 17).

Fibromyalgia (FM) is another generalized systemic chronic muscle disorder occurring primarily in women between ages 25 and 50. Unlike myofascial pain, which involves one or more specific muscles, FM is characterized by widespread pain and aching muscles throughout the body, especially in the weight-bearing muscles. The pain is accompanied by reproducible tender points at specific anatomical locations which are often confused with myofascial TrPs; both are tender to palpation, but only TrPs refer pain, elicit a jump response when palpated, and cause central excitatory effects (19). Inclusionary criteria for FM are a patient's complaint of chronic diffuse pain in all quadrants of the body for at least three months, and the presence of at least 11 of the 18 bilateral, symmetrical tender points in particular areas. Associated symptoms include chronic fatigue, a sleep disturbance, tension-type headaches, and irritable bowel syndrome. All blood tests and imaging studies are normal and diagnosis is by exclusion (15, 18).

Common disorders affecting the temporomandibular joint (TMJ) include a noninflammatory mechanical disturbance of the joint caused by a displaced articular disc (internal derangement), capsulitis, synovitis, and osteoarthritis. In its early stages, disc displacement with reduction creates a reproducible joint sound (click) present during opening and closing. Pain, when present, is perceived as a preauricular ache that often radiates to the ear, the side of the head, or the face, and is exacerbated by joint function. While many patients may demonstrate a click indefinitely without developing significant pain or dysfunction, a few patients may progress to a more advanced stage of dysfunction, including disc displacement without reduction. In its acute stage, disc displacement without reduction is characterized by an absence of clicking, signifying that the disc is still anteriorly displaced and not reducing. Severe pain in the joint, limited movement, and associated masticatory myalgia are often present. Over time, pain generally decreases and mandibular range of motion increases (19, 20).

When repetitive forces on the joint increase beyond its adaptive capacity, degenerative changes may occur in the articular surfaces. When these changes are accompanied by inflammation, the condition is referred to as osteoarthritis. Signs and symptoms of TMJ osteoarthritis usually include dull, aching pain with occasional burning in the joint and palpable joint tenderness sometimes accompanied by swelling. Crepitus, or multiple joint noises, and limited movement secondary to pain or degeneration may also eventually develop (14, 19, 21).

Of all the systemic polyarthritides, rheumatoid arthritis has the greatest propensity for affecting the TMJs, with a reported bilateral involvement of about 50%. Common TMJ complaints associated with rheumatoid arthritis include a deep, dull, preauricular pain during function, joint stiffness in the morning, clicking, crepitus, some loss in bite force, and tenderness to palpation. Progression of the disease process may cause condylar destruction and flattening of the eminence, resulting in an occlusal-facial deformity. TMJ symptoms often parallel the general disease; as the systemic disorder improves, complaints decrease. The key to the differential diagnosis is the presence of generalized symptoms in other joints of the body, including pain, swelling, morning stiffness, and loss of function along with positive laboratory findings (19, 21).

Lyme disease is a multi-organ disorder which can cause systemic effects such as headache, stiff neck, chills, fever, diffuse myalgias, malaise, and marked fatigue as well as neurological and cardiovascular abnormalities. Even though it is usually associated with large peripheral joints such as the knees, Lyme arthritis can involve the TMJ. Common symptoms include ear pain, TMJ pain, jaw claudication, and occasional palsy of the facial nerve. Because Lyme disease may mimic hundreds of other disorders, it frequently creates a diagnostic dilemma. Any complaint of TMJ pain or difficulty in chewing when accompanied by varying, unexplained symptoms should include Lyme disease in its differential diagnosis (22, 23).

The cervical spine is an integral part of the upper quarter, which is composed of the head, neck, and shoulder girdles. Any dysfunction or disorder involving one of these components has the potential to cause pain in an adjacent or functionally related area. Degenerative joint disease involving the cervical vertebrae, disc disease, rheumatic disorders, and trauma are the most frequent causes of cervical clinical syndromes. Another, often overlooked source of pain that arises from the cervical region is related to forward head posture. Forward head posture, especially when accompanied by forward shoulder position, has been associated with the development of myofascial TrPs in the sternocleidomastoid and upper trapezius muscles. Entrapment of the greater occipital nerve, causing occipital neuralgia, may also result from forward head posture (24, 25). Common symptoms related to a cervical spine disorder include neck pain and dysfunction, as well as referred pain to the head and face. When the pain is musculoskeletal in origin, it is most often described as a deep ache with sporadic episodes of sharp pain when the area is stressed. Limited range of motion of the head and cervical spine, accompanied by pain or suboccipital tenderness and pain provoked by head and neck movement, may indicate a possible cervical etiology of pain (24–26).

Neurovascular Disorders

The neurovascular pain disorders (migraine, tension-type, and cluster headaches) are common causes of chronic head and neck pain. They are a source of great concern because they can be severe and incapacitating. The pathogenesis of migraine headaches is controversial, but current research has implicated a neurogenic etiology (neurogenic inflammation) possibly causing secondary cerebrovascular changes. A wide diversity of factors are known to be capable of triggering the attacks, including diet, stress, sleep disturbances, and menstruation. The forms of migraine that the clinician is most likely to encounter are migraine with aura, formerly known as classic migraine, and migraine without aura, previously referred to as common migraine (16, 21, 51).

No laboratory or radiological tests exists for migraine; diagnosis is made on the basis of clinical features. Both forms of migraine have a hereditary predisposition, begin at a young age, primarily affect women, and occur with a variable frequency. Typical attacks may last from 4–72 hours, are usually unilateral, and are characterized as having pain that is pulsating with moderate to severe intensity. Sometimes the pain may be bilateral, especially in children, and non-pulsatile. Migraine is commonly associated with symptoms of nausea and/or vomiting, photophobia, and phonophobia. These headaches often begin immediately after awakening or soon thereafter. Routine physical activity aggravates them. Patients typically are unable to function and just wish to lie down in a dark room. Clinically, migraine with aura is similar to migraine without aura except that the attack is preceded by some symptoms which start from several minutes to an hour before the onset of the headache. This prodrome is characterized by visual disturbances, such as flashing lights and colors, zigzag patterns, or blind spots and, on occasion, numbness and tingling more on one side of the face and arm (21, 51).

Although cluster headaches are relatively uncommon, it is one of the most severe forms of headache and facial pain, and has been referred to as a "suicide headache" (7). Cluster headaches predominantly affect males, with a clinical male-to-female ratio of 5:1. There are two types: an episodic type, where the attacks of pain occur during a time of susceptibility, known as the cluster period, which may last for weeks or months; and a less common chronic form, where there is an absence of pain-free remission periods (9, 11). A cluster attack may last from 15 minutes to 3 hours, and can occur from once every other day to as often as 8 times per day. Frequently, the patient is awakened from sleep by the attack. Cluster pain is excruciating and has a boring, sharp, or burning quality. A typical attack is unilateral in location, involving the periorbital area, often with radiation to the ipsilateral temple and maxilla, including the teeth. It is almost always associated with autonomic symptoms such as lacrimation from the eye on the affected side, nasal congestion, rhinorrhea, forehead and facial sweating, ptosis, and eyelid edema. Another form of cluster, paroxysmal hemicrania, occurs mostly in females and features similar attacks that are shorter and more frequent (9, 20, 36).

Tension-type headache, previously called tension headache or muscle contraction headache, is divided into two major categories: episodic and chronic. The episodic tension-type headache lasts for 30 minutes to one week while the chronic form occurs with a frequency of at least 15 days a month for at least 6 months. Pain is usually described as a steady, dull, aching sensation of mild to moderate intensity, but throbbing pain may occasionally be present. The headaches occur primarily in the bifrontal occipito-nuchal and bitemporal areas, and are often associated with stiffness and tenderness of the neck. Photophobia or phonophobia may also be part of the headache complaint in some patients. Unlike migraine, physical activity does not worsen the pain, and nausea is usually absent (27–33). The chronic type may evolve from the episodic type and shares similar clinical features. Tension-type headaches must be differentiated from migraine without aura, referred pain from myofascial trigger points, cranial arteritis, and TMD, especially when bitemporal pain is a chief complaint. Some authors use another term, chronic daily headache, to describe a daily headache with clinical features primarily of a tension-type headache with superimposed episodes of migraine. Chronic daily headache is believed to represent a transformation of episodic migraine syndrome. These symptoms, called by some the rebound headache, may also result from frequent and excessive use of prescription and over-the-counter medications (34–37).

Neurological Disorders

Neurogenic or neuropathic pain is due to a functional abnormality within the peripheral nervous system and does not involve noxious stimulation. Neuropathic pain may be divided into two main categories: paroxysmal and continuous disorders (1, 2). The most common paroxysmal facial pain is trigeminal neuralgia (TN), which occurs unilaterally in the distribution of one or more divisions of the trigeminal (V) nerve, usually the mandibular or maxillary. The time pattern of the pain is episodic, lasting for a few weeks to approximately a month, followed by a remission period of several months or years. When a pathological causal component (e.g., tumor, aneurysm, multiple sclerosis) can be identified, TN is classified as symptomatic; if a causative factor cannot be found, the condition is known as idiopathic TN. Onset is usually after age 40, affecting women more than men in a 3:2 ratio (1, 38, 39). TN can present as pain originating in the skin of the face, part of the mandible or maxilla, or frequently as a toothache, leading to a misdiagnosis of dental pathology. Patients describe the pain as "the worst pain I've ever had" and trace the pain along the path of the affected nerve (12). About 50% of patients have trigger zones on the face, which when lightly touched can precipitate the characteristic pain, causing patients to avoid normal activities such as eating, talking, or washing the face (39, 40). A careful history, along with a lack of obvious dental cause, should rule out odontogenic pain. TN must also be differentiated from TMD. Normal mandibular function can trigger the pain when trigger zones are present, and tic-like cramps or involuntary spasms of the facial muscles known as tic douloureux, often accompany a typical pain attack. A local anesthetic block of the involved nerve will almost always arrest the pain of TN, but will not usually affect TMD symptoms. This procedure, in combination with a careful history, should lead to the correct diagnosis (41, 42).

Continuous neuropathic disorders are characterized by constant, unremitting pain of variable intensity. The pain usually has a burning quality and may be accompanied by paresthesia (abnormal sensation) or dysesthesia (unpleasant sensation). A most perplexing example of a continuous neuropathic disorder is atypical odontalgia (AO), also known as phantom tooth pain. AO refers to persistent pain in apparently normal teeth and surrounding alveolar bone, or at extraction sites from which teeth have been removed because of pain (1, 43, 44). AO occurs mostly in women in their early forties, frequently affecting molars and bicuspids. Common characteristics are constant toothache or dental socket pain which can spread to other teeth, chronicity greater than four months, normal radiographs, and no clinically observable cause. The pain is usually described as dull, aching, throbbing or burning, and may be accompanied by an increased sensitivity to pressure over the affected area, and a feeling of dysesthesia. Standard dental diagnostic procedures such as pulp testing, thermal testing, and percussion give inconclusive results, and diagnostic nerve blocks are usually equivocal, leading to a diagnosis by exclusion (41, 43, 44). Current thinking seems to favor the concept of deafferentation initiated by some form of trauma to the offending tooth, such as endodontic therapy, endodontic surgery, periodontal surgery, tooth extraction, traumatic injury, or implants. "Deafferentation" refers to the partial or total loss of a sensory nerve supply to a particular body region. Following nerve injury, there is usually a feeling of numbness. However, in a small percentage of cases, a paradoxical situation of spontaneous pain may develop in the area of the diminished sensation. Onset of deafferentation pain can be delayed after the initial tissue damage, is highly resistant to treatment, and can spread to other orofacial structures (44–47).

Clinicians should also be aware of two other continuous neuropathic disorders affecting the trigeminal nerve: traumatic neuromas and trigeminal neuritis. When a peripheral nerve is partially or completely severed as a result of trauma injury, it attempts to repair the damage by regenerating. When this attempt is unsuccessful, a disorganized, intertwined mass of nervous tissue can result. Neuromas are characterized by deep, aching, burning pain induced by compression or stretching at the site of injury (41, 48). Trigeminal neuritis is due to inflammation of the peripheral branches of a nerve trunk as a result of trauma, bacterial or viral infection, or toxic causes. The pain is described as burning, bright, or stimulating, and is usually accompanied by a feeling of paresthesia or anesthesia in the distribution of the affected nerve. An example of neuritis affecting the facial nerve is Bell's palsy, which usually results from compression of the nerve inside the facial canal or stylomastoid foramen. Although Bell's palsy is not usually painful, it often causes facial paralysis and an obvious facial asymmetry (49). It should be noted that the sympathetic nervous system, specifically sympathetic efferent activity, may be involved in various deafferentation pain disorders as well as other neuropathies related to trauma. Sympathetic involvement should be suspected when the pain spreads, is refractive to treatment, and is accompanied by extreme sensitivity to light touch (allodynia), and autonomic symptoms (50, 51).

Psychological Disorders

Pain from psychological causes includes somatization disorder, conversion hysteria pain, and hypochondriasis, all of which exhibit the clinical characteristics of psychogenic pain. Because most patients with chronic pain have psychological problems (e.g., depression, anxiety), their pain is incorrectly labeled as psychogenic in origin. Chronic pain behavior must not be confused with psychogenic pain. While psychogenic pain arises from an emotional or personality disorder that fulfills psychiatric diagnostic criteria, the psychological problems found in chronic pain patients are the result of the pain rather than its cause (5, 52, 53).

Conclusion

An organized approach is necessary to assess and diagnose orofacial pain, since multiple medical and dental specialties are involved in the management of patients. The clinician must be able to correctly diagnose and treat the patient accordingly, or refer the patient to the appropriate specialist.

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Oral Manifestations of Nutrient Deficiencies

Riva Touger-Decker, M.S., Ph.D.

Abstract

There is a synergistic relationship between nutrition, oral health, and disease. Nutrient deficiency may result in oral symptomatology. Oral conditions and systemic diseases associated with oral manifestations may lead to nutrient deficiencies as a consequence of compromised mastication and swallowing, pain, or discomfort. Health professionals must recognize the manifestations of these deficiencies, consider their causes and provide early treatment to prevent further compromise in nutrition status and to promote optimal nutrition, oral and overall health. **Key Words:** Nutrition, oral cavity, oral manifestations of nutrient deficiencies.

Introduction

The relationship between nutrition and oral health is multifaceted. Nutrition has both local and systemic impacts on the oral cavity (1). While diet and eating patterns have a local effect on the teeth, saliva and soft tissues, the systemic impact of nutrition also has considerable implications and it too merits assessment as a component of comprehensive care. The systemic effect is the impact of the nutrients consumed as they assume their biological functions in relation to the development and maintenance of the extra- and intraoral structures and secretions (1, 2). An adequate supply of nutrients is essential to the growth, development, and maintenance of tissues, effectiveness of the immune system, prevention of cell damage and, in general, to increased resistance to many chronic, and some infectious diseases (1). The oral cavity is often one of the first sites where nutrient deficiencies can be clinically noted.

Clinical manifestations of nutrient deficiencies can have a significant impact on the function of the oral cavity. Functional properties of the oral cavity include taste, salivation, mastication, and swallowing food. Any alterations in the structure and function of the oral cavity may compromise intake and contribute to the development of a nutrient-deficiency state. When the associated oral structures are affected, these alterations may be compounded even further, leading to subsequent inadequate dietary intake and compromised nutrition status. This article will focus on the development of nutrient deficiencies that have oral manifestations, the relationship of malnutrition and oral diseases, and nutrition management strategies. The relationship of systemic diseases associated with nutrient deficiencies and their oral manifestations will also be discussed.

Development of Nutrient Deficiencies

Nutrient deficiencies result from an imbalance of supply and demand, that is, when the supply of nutrients is inadequate to meet the demands of the body (3). The imbalance may result from one of three primary causes: inadequate intake, impaired digestion and absorption,

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From the School of Health Related Professions, New Jersey Dental School, University of Medicine and Dentistry of New Jersey, Newark, NJ. Assistant Professor and Program Director of Clinical Nutrition, and Clinical Assistant Professor.

Address correspondence to Riva Touger-Decker, M.S., Ph.D., University of Medicine and Dentistry of New Jersey - SHRP, Department of Primary Care, 65 Bergen Street, Room 158, Newark NJ, 07107-3001.

or increased losses. A deficiency of any one nutrient may in turn contribute to subsequent deficiencies of other nutrients. Clinical manifestations of deficiencies occur once tissue stores are depleted. They may present as symptoms reported by patients as well as by physical observations notable on examination (3). Extra-oral manifestations include alterations in appearance and integrity of the skin, while intra-oral manifestations may be reflected in altered integrity and appearance of the teeth, soft tissue, and tongue (Table 1). Left untreated, deficiency symptoms may progress to deficiency diseases.

Once a negative nutritional imbalance has occurred, depletion of tissue levels and body stores occur. This is evidenced by depleted serum levels of nutrients and/or altered response to diagnostic tests. Serum levels may not always reflect nutrient stores. Once body stores are depleted, biologic and physiologic performance and cell functions of those cells dependent on the specific nutrient become altered. Following altered biologic and physiologic performance, clinical manifestations typically appear in the development of a deficiency (4). Once clinical symptoms become apparent, morbidity and mortality rise.

A first step in assessing nutrient deficiencies is to determine the cause (Table 2). A careful review of the medical history and presenting symptoms with physical examination is critical to detecting deficiency conditions and possible etiologies. Illness and infection can increase nutrient needs and thereby contribute to a deficiency. Bacterial and fungal infections in the oral cavity may alter tissue integrity, increase nutrient utilization, and compromise intake. The end result is twofold, with increased needs and decreased dietary intake. Inflammatory bowel disease may cause deficiencies of water soluble vitamins including B6, B12, folate, trace elements, calcium, zinc, and magnesium due to altered digestion and absorption. Inadequate intake may be due to functional difficulties such as poor dentition, tooth loss, ill-fitting dentures, xerostomia, and systemic disease, as well as psychosocial problems including lack of income, homelessness, depression, and anorexia. A prolonged poor dietary intake due to ill-fitting dentures and difficulties with mastication has been shown to result in inadequate intake of zinc, calcium, and B6 (5).

TABLE 1
Nutrition Risk Factors to Consider in Physical Examination

Oral Cavity Area	Clinical Manifestation	Nutrient Deficiencies
Face	Malar pigmentation	Niacin, B vitamins
	Bitemporal wasting	Protein-energy malnutrition
	Nasolabial seborrhea	Niacin, riboflavin, B6
	Edema	Protein deficiency
	Lack of color	Iron, malnutrition
Lips	Cheilosis	Niacin, B6, riboflavin
	Angular fissures	Niacin, B6, riboflavin, iron
Gingiva	Spongy, bleeding, abnormal redness	Vitamin C
Tongue	Glossitis (red, raw, fissured)	Folate, niacin, iron, B6, B12
	Pale, atrophic, smooth/slick (filiform papillary atrophy)	Iron, folate
	Magenta color	Riboflavin

TABLE 2
Causes of Nutritional Deficiencies from A to Z

A norexia
B ulemia
C orticosteroids
D iet
E thanol
F ungal infections
G one to a nursing home
H omelessness
I nflammatory bowel disease
J ust tea and toast diet
K aposi's sarcoma
L axative abuse
M edications
N o sunlight
O ral contraceptives
P oor dentition
Q uackery
R adiation therapy
S oft tissue abnormalities
T ooth loss
U lcerations in the mouth
V itamin abuse
W eight loss
X erostomia
Y ellow fever
Z ero money for food

Malnutrition and Periodontal Disease(s)

Periodontal diseases, a group of infectious diseases which are mostly chronic, affect the supporting tissues of the teeth. Compromised host defense responses associated with malnutrition may make the periodontium more susceptible to infectious organisms that are a normal component of the oral flora. The acute phase protein response to tissue injury is impaired to varying degrees in malnourished individuals. During periods of malnutrition, the magnitude of the inflammatory response is limited, resulting in an impaired host response (6, 7). This could result in a greater amount of periodontal destruction, leading to a compromised dentition. Along with dental and/or pharmacological treatment of the underlying local etiologic factors, nutritional management goals focus on provision of adequate calories, protein and nutrients to promote tissue repair, restoration of the host defense mechanisms, and overall well being. Controversy exists regarding the relationship between nutrition status and periodontal disease (6–11). It is important to note that although some nutritional deficiencies (notably vitamin C and folate) can alter the disease process, periodontal diseases are not caused by these deficiencies nor can they be cured by nutrient supplementation alone (8, 9).

Gingivitis, a form of periodontal disease, refers to inflammation of the soft tissue component of the periodontium. There are multiple causes of gingivitis including: chronic diseases such as diabetes; medications including phenytoin and calcium channel blockers; pregnancy; and nutrient deficiencies. Spongy, red, bleeding and painful gingiva are also noted in scurvy, an advanced vitamin C deficiency disease. In severe gingivitis, the easy bleeding and soreness of the gingiva may make eating difficult and contribute to poor intake. Soft, nonirritating, temperate and mildly flavored foods and fluids should be provided to meet energy and nutrient needs. Historically, vitamin A deficiency (12) was cited as a cause of periodontal disease. There are no research studies published within the past decade showing that a deficiency of this vitamin is the cause of gingivitis. Clinical manifestations of folate deficiency, however, may include gingivitis.

Oral Manifestations of Nutrient Deficiencies

While nutrient deficiencies may contribute to abnormal color, topography, size and sensations in the oral cavity (13), other causes of abnormal findings such as glossodynia and glossopyrosis (painful and burning tongue and soft tissue), dysgeusia (altered taste), angular cheilitis (painful, dry cracked corners of the mouth), and changes in appearance and texture of the tongue must also be evaluated. Abnormal findings may reflect oral manifestations of a myriad of systemic diseases, medications, disorders unique to the oral cavity, or a nutrient deficiency (14). Three possible categories of etiologies include: oral manifestations of a nutrient deficiency, oral manifestations of a systemic disease that impacts on diet and nutrition status, and local oral disorders that interfere with dietary intake (Table 3).

Angular Stomatitis and Cheilosis

Angular stomatitis (painful fissures at the corners of the mouth) and cheilosis (dry scaling of the lips and corners of the mouth) are common findings in riboflavin deficiency. Similar findings may be noted with niacin and B6 deficiency states. The similarity of these findings may be due to riboflavin's role in B6 and tryptophan (which is converted to niacin) metabolism. Angular stomatitis, however, may be associated with iron deficiency anemia (15). Angular cheilitis, however, is often associated with fungal infections, lip-sucking, and dehydration (16). Treatment must focus on correcting the deficiency state and providing adequate energy, protein, fluids and nutrients to promote healing. When angular cheilitis is due to opportunistic infections brought on by decreased resistance secondary to nutrient deficiencies, treatment should focus on antifungal therapy, correction of the nutrient deficiency, and diet modification to make eating a more comfortable experience. Temperate, nonspicy foods and fluids should be used to avoid further irritation to the lips and mouth. At least 6–8 cups of fluid per day should be encouraged. Individuals with angular stomatitis or cheilosis may experience difficulty and pain when they try to open their mouths wide to laugh or eat. They will lick their lips repeatedly with their tongues to moisten them, a habit that should be discouraged to prevent further irritation and infection.

Changes in the Tongue

There are several changes that can occur on the tongue during nutrient deficiency states (Table 1). A painful, magenta colored, atrophic, smooth tongue is noted during a riboflavin deficiency. Glossitis may also be evidence of a vitamin B6, folate or B12 deficiency. However, during a chronic folate deficiency, the tongue papillae will become atrophied, resulting in a shiny, smooth surface appearance. Glossitis with loss of filiform papillae may also be seen in individuals with iron-deficiency anemia. A niacin deficiency results in a raw beefy, bright red, swollen, and painful tongue. Glossodynia may also be present in diabetes, resulting in painful mastication and swallowing. Medical nutrition management focuses on correcting the cause of the deficiency, provision of adequate calories, protein and nutrients for healing, and a nonirritating diet consisting of liquids and soft solid foods moderate in temperature and mild in taste.

Altered Taste

Hypogeusia, or diminished taste, is noted in zinc deficiency. Other non-deficiency state

TABLE 3

Abnormal Oral Findings Associated With Local and Systemic Disease

Clinical Feature	Associated Finding	Associated Disorder	Nutritional Considerations
Xerostomia	Dental caries Candidiasis Dysphagia	Drug-induced xerostomia Sjîgren's syndrome	Push fluids, evaluate cariogenicity of diet Modify food consistency and choices to reduce pain (limit spicy, hot, acidic, and seasoned foods)
	Burning mouth and tongue	Connective tissue disorders	Evaluate masticatory efficiency and modify food choices
		Diabetes	Evaluate glucose control, modify diet
Burning mouth and tongue	May be with or without associated erythema, edema (stomatitis)	Anemia Diabetes Candidiasis	Determine etiology of deficiency Determine cause of poor glucose control, modify diet, evaluate cariogenicity, evaluate for dysgeusia, dysphagia
	Glossitis	R/O deficiency of iron, folate, B6, B12, niacin and/or riboflavin	Determine etiology; treat with diet and/or supplements as needed
	Pale, atrophic, smooth Tongue	R/O deficiency of iron, folate and B12	See above
Angular fissures of the lips	Dry, cracked lips	R/O niacin, riboflavin, B6, iron deficiency, dehydration	Determine etiology
Thrush	White patches on palate or buccal mucosa that can be rubbed off	Candiadiasis	See above
Difficulty biting/chewing food	Partial or total edentulism Lack of occlusion Ill-fitting dentures		Modify diet consistency Loss of anterior occlusion- modify for difficulty biting Loss of posterior occlusion- modify for difficulty chewing

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causes of altered taste include radiation to the head and neck, diabetes mellitus, and Sjögren's syndrome. Taste tests should be conducted to determine which taste sensations remain. Diets should be tailored to highlight the remaining taste sensations and limit those foods or flavorings which are offensive. Dysgeusia refers to altered taste, which may also affect appetite and intake.

Xerostomia

Xerostomia is rarely due to a nutrient deficiency. It may be seen in severe vitamin A deficiency states (13) and in protein calorie malnutrition. The primary causes of xerostomia (Table 4) include medications, Sjögren's syndrome, diabetes mellitus, and radiation to the head and neck (17). Altered taste sensations are frequently reported by individuals with xerostomia (18). Artificial salivas may be used. However, they may also contribute to altered taste. Inadequate salivary flow can also contribute to oral infections, including dental caries and glossopyrosis (17, 18). Moist, non-spicy foods and temperate fluids should be provided; fluids are particularly important at mealtime. Individuals with xerostomia should be encouraged to use tart or citrus flavored sugar-free gums and candies in place of the sugared varieties to increase salivary flow. Oral hygiene is very important to reduce risk of dental caries, particularly following meals and snacks.

Systemic Diseases Associated with Oral Manifestations and Nutrient Deficiencies

The comprehensive management of patients with systemic diseases requires a coordinated team approach of the physician, dentist, registered dietitian (R.D.) and other health professionals. Medical, dental and nutritional management of the disease and associated sequelae are essential to maximize disease management and minimize the often harmful side effects of disease. Medical management should include reinforce-

TABLE 4
Causes of Xerostomia

Disease conditions:

- Type 1 and 2 Diabetes
- Sjögren's Syndrome
- AIDS
- Rheumatoid arthritis

Medications:

- Anticonvulsants
- Antihistamines
- Diuretics
- Muscle relaxants
- Antiviral agents
- Anti-anxiety agents
- Antidepressants
- Narcotics
- Antihypertensive agents
- Tranquilizers
- Serotonin update inhibitors

ment of medical nutrition therapy and oral hygiene principles provided by the R.D. and dentist respectively. Medical and dental professionals should screen and identify patients at nutrition risk and refer patients to an R.D. for comprehensive medical nutrition therapy.

Inflammatory Bowel Disease

Inflammatory bowel diseases, including ulcerative colitis and Crohn's disease, may cause deficiencies of vitamin B6, B12, folate, iron, zinc, magnesium and calcium. The impact of these deficiencies on the oral cavity has been discussed in the previous section. In addition, Crohn's disease may cause a painful aphthous stomatitis which can compromise eating and nutritional status. Determination of the cause of the deficiency and appropriate management is important to prevent further deterioration in nutrition status and compromised eating ability.

Diabetes

Diabetes mellitus is a systemic disease associated with delayed wound healing and oral manifestations which may alter nutrient intake and compromise nutrition status. Poorly controlled diabetes mellitus is associated with glossodynia, xerostomia, candidiasis, gingivitis, periodontitis and altered taste (17, 19, 20). Up to 30% of individuals over the age of 19 with type 1 diabetes have periodontal disease (20). Good oral health is important in order for individuals to be able to consume a diet adequate for maintaining glycemic control. Good glycemic control through diet is essential to restore oral and overall health, and maximize patient response to dental procedures including surgery, denture placement and operative care. Individuals with diabetes should be referred to an R.D. for medical nutrition therapy. Diet counseling is extremely important prior to and following surgical or treatment procedures which may alter eating ability.

Renal Disease

Renal disease has significant dietary, nutritional and oral implications. Significant restriction of dietary protein, sodium, potassium, phosphorus and fluid intake is necessitated for management of patients with renal failure. Meanwhile, calorie and calcium intake, as well as other water soluble vitamins and iron, need to be increased to promote and maintain desirable body weight and nutritional status. Iron deficiency anemia, osteomalacia, and deficiencies of other water soluble vitamins may occur. Dental sequelae including ulcerative stomatitis, xerostomia, and urea in the saliva may further compromise nutritional status and dietary intake. The high simple carbohydrate diet followed by individuals with end-stage renal disease combined with the presence of xerostomia increases risk of tooth decay. Rinsing the mouth or brushing teeth following all meals and snacks should be encouraged to minimize caries risk factors.

Human Immunodeficiency Virus (HIV)

HIV-associated oral conditions include Kaposi's sarcoma, fungal, bacterial and viral infections, ulcerative stomatitis, and periodontal disease (21). These problems are all associated with functional limitations on eating ability, contributing to diminished oral intake and consequent malnutrition. In order to meet energy and nutrient needs, diets need to be individually tailored in texture, temperature and consistency to minimize pain and maximize intake. Appetite stimulants may be used to combat anorexia and promote weight gain. Oral nutrition supplements may be needed to meet the increased caloric and protein needs of the HIV^+ patient.

Head and Neck Cancer

Head and neck cancer therapies are associated with painful oral conditions that may compromise intake. Surgeries may compromise mastication, increase energy and nutrient needs and permanently affect eating ability. Radiation to the oropharyngeal region may result in xerostomia, altered taste, tooth loss, stomatitis and muscle fibrosis. These complications, combined with anorexia, may lead to nutrient deficiencies and malnutrition. The vicious cycle of oral manifestations secondary to therapy, nutrient deficiencies and increased needs for nutrients is quite apparent in this population. Medical nutrition therapy is critical to meet energy, nutrient and fluid needs with a diet tailored to the taste, consistency and temperature needs of the individual. If needs cannot be met orally, enteral tube feedings should be considered to prevent further deterioration in nutrition status, replete deficiency states, and restore overall nutritional health.

Transplant Patients

Oral health and nutrition status must be monitored in the management of the transplant patient. The immunosuppressive medications and steroids have oral- and nutrition-related sequelae including impaired glucose tolerance, osteoporosis, osteopenia and compromised integrity of the oral cavity soft tissue. Vitamin and trace element supplements are prescribed to promote repletion of body stores. Calcium in particular is needed to prevent osteoporosis associated with steroid therapy.

Conclusion

The health care paradigm has shifted; physicians, dentists and registered dietitians, as well as other health professionals, are increasingly responsible for the conduct of comprehensive health screening, discipline-specific management and referral to the appropriate provider for more comprehensive care. Nutrition risk evaluation is part of the scope of practice of all health professionals; early detection of risk for nutrient deficiencies during medical or dental exams, recognition of oral manifestations of nutrient deficiencies and other diseases with referral to and the collaboration with the registered dietitian in the provision of nutrition therapy is the responsibility of all health-care providers (22–24). Registered dietitians have also assumed the responsibility of incorporating a comprehensive approach to patient assessment and management in addition to providing medical nutrition therapy (24).

Further research is necessary to understand more fully the synergy between nutrition and oral conditions in health and disease. Given the diversity of information and misinformation available concerning nutrients, it is incumbent upon professionals to understand the relationships between nutrients and oral manifestations if they are to properly diagnose and triage their patients as well as review the scientific basis and research cited to support the claims and advise patients accordingly. Nutrition can only cure malnutrition. More is not always better; toxicities, particularly with certain trace elements and fat soluble vitamins, are possible.

There is a clear synergy between oral health and nutrition; nutrition plays a crucial role in maintaining the integrity and normal function of the oral cavity (24). Nutrient deficiencies and oral manifestations of disease which alter that integrity contribute to compromised dietary intake, resulting in deficiency states, malnutrition and poor wound healing following procedures or surgeries. It is the responsibility of all health professionals to be alert to risk factors for and clinical signs of nutrient deficiencies in their patient populations. Physicians, dentists, registered dietitians and other health professionals must collaborate in the provision of comprehensive patient care. Collaborative approaches to patient assessment, management and referral are critical to the emerging role of today's health care professional.

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Periodontal Disease:

An Overview for Physicians

Kim E. Fenesy, D.M.D.

Abstract

Periodontitis is now seen as resulting from a complex interplay of bacterial infection and host response, often modified by behavioral factors. There has been a fundamental change in the prevailing periodontal disease model of the 1960s, which suggested that the susceptibility to periodontitis increases with age, and that all individuals are susceptible to severe periodontal disease. More recent research has changed the belief in universal susceptibility to the current view that only some 5–20% of any population suffer from severe generalized periodontitis, and that only moderate disease affects a majority of adults.

One major risk factor is smoking, as there is now a clear association between smoking and periodontal disease independent of oral hygiene, age, or any other risk factor.

In human periodontitis, there is no simple, direct pathogen-disease link. There are three pathogens that have a strong association with progressive periodontal disease: *Actinobacillus actinomycetemcomitans,* spirochetes of acute necrotizing gingivitis, and *Porphyromonas gingivalis.* These pathogens may be the cause of continued loss of periodontal attachment in all periodontal disease classifications despite diligent periodontal therapy. This loss of attachment, or destruction of the periodontal ligament and loss of adjacent supporting bone, is seen in adult periodontitis, as well as in early-onset periodontitis, which affects young persons who otherwise appear healthy. The three forms of early-onset periodontitis are prepubertal periodontitis, localized and generalized juvenile periodontitis, and rapidly progressive periodontitis. They are distinguished from adult periodontitis by the age of onset of the disease, the rapid rate of disease progression, manifestations of defects in host response, and the composition of the subgingival microflora. Prepubertal periodontitis is associated with attachment loss around teeth of the deciduous and/or permanent dentition, and is often associated with severe congenital defects of hematological origin, and alterations in neutrophil chemotaxis function.

Periodontitis may also be associated with systemic conditions such as metabolic disorders (diabetes mellitus, female hormonal alterations), drug-induced disorders, hematologic disorders/leukemia, and immune system disorders. These systemic disorders have been documented as capable of affecting the periodontium and/or treatment of periodontal disease.

In order to rationally treat and prevent periodontal disease, we need to know the etiologic agents for specific patients, and the mechanism of bacterial pathogenesis in periodontitis. In systemic diseases in which the periodontal tissues are affected as well, early detection and carefully managed therapeutics with the physician and periodontist working together may prove beneficial to the patient's general health and quality of life. **Key Words:** Adult periodontitis, early-onset periodontitis, prepubertal periodontitis, juvenile periodontitis, rapidly progressive periodontitis, *P. gingivalis, A. actinomycetemcomitans*

Periodontitis is now seen as resulting from a complex interplay of bacterial infection and host response, often modified by behavioral factors (1). Advances in research over recent years have led to a fundamental change in the periodontal disease model. The prevailing model of periodontal disease in the 1960s suggested that all individuals are susceptible to severe periodontitis, that gingivitis progresses to periodontitis with consequent bone and tooth loss, and that the susceptibility to periodontitis increases with age (2–4). All aspects of this model are under strong challenge from the results of more recent research. The belief in universal susceptibility has given way to the current view that only some 5%–20% of any population suffer from severe generalized periodontitis, even though moderate disease affects a majority of adults (5).

Risk factors, such as smoking, have a profound effect on the predisposition to periodontal disease. There is now a clear association between smoking and periodontal diseases, independent of oral hygiene, age, or any other risk factor (6). Studies have shown that there is no difference

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From the Department of Oral Pathology, Biology and Diagnostic Sciences, New Jersey Dental School, University of Medicine and Dentistry of New Jersey, Newark, NJ.

Address correspondence to Kim E. Fenesy, D.M.D., University of Medicine and Dentistry of New Jersey, New Jersey Dental School, Department of Oral Pathology, Biology and Diagnostic Sciences, Room C-829, 110 Bergen Street, University Heights, Newark, NJ 07103-2400.

between smokers and nonsmokers in amounts of plaque accumulation, nor in the prevalence of the principal bacteria which are considered pathogenic for periodontitis (7, 8). What smoking appears to do is suppress the vascular reaction which follows gingivitis (9). In effect this is a masking effect on the signs of inflammation. Smoking may also be a factor in the association between refractory periodontitis and a polymorphonuclear leukocyte defect in the peripheral blood. There is also some evidence that smoking is associated with osteoporosis; the link with dental alveolar bone loss is currently under study (10, 11).

In human periodontitis, there is no simple, direct pathogen-disease link. More than 500 bacteria have been identified within periodontal pockets (12). Moreover, it is impossible to accurately associate the complex microbiota in a periodontal lesion, in a given patient, with the bacterial species that may have contributed to development of that lesion. Much evidence suggests, however, that a finite set of pathogenic microbiota, sometimes working alone, or in combinations, cause periodontal diseases in humans. Most pathogens associated with periodontal lesions are Gram-negative anaerobic rods, while some pathogens are also Gram-positive facultative and anaerobic cocci and rods, and Gram-negative facultative rods. Due to this mixed variety of pathogens, and their variability in sensitivity to antibiotics, simplistic antimicrobial chemotherapy is problematic. Three pathogens have an especially strong association with the presence of progressive periodontal disease: *Actinobacillus actinomycetemcomitans*, spirochetes of acute necrotizing gingivitis, and *Porphyromonas gingivalis* (13). One potential virulence factor recently ascribed to *P. gingivalis* and *A. actinomycetemcomitans*, which is shared by a number of respiratory and enteric pathogens, is the ability to enter mammalian cells (14). These pathogens are very often the cause of continued loss of periodontal attachment despite diligent conventional mechanical periodontal therapy, as well as causing refractory periodontitis (15), localized juvenile periodontitis, and other types of early-onset periodontitis (16). Patients who suffer from these conditions may benefit from antibiotic therapy.

Evaluation of the patient's periodontal status requires obtaining a relevant medical and dental history and conducting a thorough clinical and radiographic examination, with evaluation of extraoral and intraoral structures. A medical history should be taken and evaluated to identify predisposing conditions that may affect treatment, patient management and outcomes. Such conditions include, but are not limited to, diabetes, hypertension, and pregnancy. Factors which may also play a role in treatment outcome are smoking, substance abuse and medications.

Adult periodontitis is defined as inflammation of the gingiva and the adjacent dental attachment apparatus (17). The disease is characterized by loss of clinical attachment due to destruction of the periodontal ligament and loss of the adjacent supporting bone (18). As mentioned previously, dental plaque bacteria play a key role in what is now understood to be the complex process by which the common types of oral diseases occur — dental caries and periodontal diseases (18). As with other infectious diseases, there is a balance between the host immune responses on one hand, and the microbial pathogenesis on the other hand. In health, host immune responses are sufficient to hold in check the pathogenic potential of both the normal resident microbial flora and exogenous microbial pathogens. Infectious diseases such as periodontal disease occur when this equilibrium is disturbed.

Clinical features may include combinations of the following signs and symptoms: edema, erythema, gingival bleeding upon probing, and/or suppuration (19) (Fig. 1). Adult periodontitis with slight to moderate destruction is characterized by a loss of up to 1/3 of the supporting periodontal tissues; a loss of over 1/3 of the periodontal supporting tissues is seen in advanced adult periodontitis. Radiographic evidence of bone loss is apparent in advanced adult periodontitis, and may be evident in adult periodontitis with slight to moderate destruction. Adult periodontitis with slight to moderate or advanced loss of periodontal supporting tissues may be localized, involving one area of a tooth's attachment, or more generalized, involving several teeth or the entire denti-

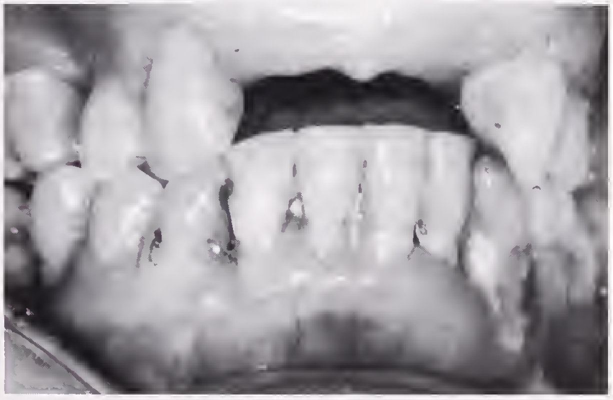

Fig. 1. Adult periodontitis — heavy deposits of plaque and calculus resulting in inflammation and bone loss.

tion (19). A patient may simultaneously have areas of health and adult periodontitis with slight, moderate, and advanced destruction.

The therapeutic goals of periodontal therapy are (1) to alter or eliminate the microbial etiology and contributing risk factors for periodontitis, thereby arresting the progression of disease and preserving the dentition in a state of health, comfort, and function with appropriate esthetics; and (2) to prevent the recurrence of periodontitis (19). In addition, regeneration of the periodontal attachment apparatus, where indicated, may be attempted.

Clinical judgment is an integral part of the decision-making process. Many factors affect the decisions for appropriate therapy(ies) and the expected therapeutic results. Patient-related factors include systemic health, age, compliance, therapeutic preferences, and patient's ability to control plaque. Other factors include the clinician's ability to remove subgingival deposits, prosthetic demands, and the presence and treatment of teeth with more advanced adult periodontitis. In general the treatment should include oral hygiene instruction, and reinforcement and evaluation of the patient's plaque control; mechanical therapy, i.e., supra- and sub-gingival scaling and root planing to remove microbial plaque and calculus; and control of other local factors. If this initial therapy resolves the periodontal condition, supportive periodontal therapy should be scheduled at appropriate intervals; but if the periodontal condition is not resolved, periodontal surgery should be considered to correct anatomic defects, and/or to regenerate hard and soft tissues.

Early-onset periodontitis encompasses distinct types of periodontitis that affect young persons who, in most cases, otherwise appear healthy. Early-onset periodontitis may be distinguished from adult periodontitis by the age of the disease onset, the rapid rate of the disease progression, manifestations of defects in host response, and composition of the associated subgingival microbial flora (19). Early-onset periodontitis includes three forms: prepubertal periodontitis, localized and generalized juvenile periodontitis, and rapidly progressive periodontitis (20).

There is evidence that genetic factors influence susceptibility to the different forms of early-onset periodontitis. However, it is unlikely that a specific gene will be identified as causing enhanced disease susceptibility. It is more likely that the genetic influences are as multifactorial as the diseases themselves, and a complex interplay between genetically determined host responses and environmental challenges may determine whether disease is present (21).

Prepubertal periodontitis is associated with attachment loss (gingival pocket formation and radiographic evidence of bone loss) around teeth of the deciduous and/or permanent dentition (22). It can occur between the time of tooth eruption and the beginning of puberty. As a consequence of this destruction, exfoliation of the deciduous teeth starts prior to the eruption of the permanent dentition. Prepubertal periodontitis may occur in either generalized or localized forms. The generalized form is most frequently associated with severe congenital defects of hematological origin and is usually accompanied by alterations in neutrophil chemotaxis function. Some forms of prepubertal periodontitis can be considered to be complications of a severe systemic disorder, such as acrodynia, cementopathia, Chédiak-Higashi syndrome, chronic neutropenia, histiocytosis X, HIV infection, hypophosphatasia, leukemia, Papillon-Lefèvre syndrome, and fibrous dysplasia (23) (Fig. 2). The localized form is usually not associated with a systemic disorder, and presently there is no evidence that localized prepubertal periodontitis will carry over to the permanent dentition.

In most reported cases of generalized prepubertal periodontitis with congenital defects of hematologic origin, the disease could not be arrested unless all the teeth were extracted (23). There are very few reports concerning the treatment of localized prepubertal periodontitis in which aggressive treatment consisted of extraction of hopeless teeth, scaling and root planing, daily subgingival irrigation, and antibiotic coverage. In general, the data support conservative supportive treatment of localized prepubertal peri-

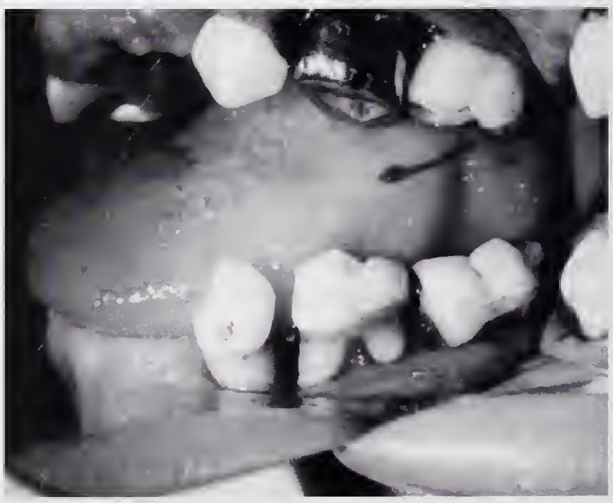

Fig. 2. Prepubertal periodontitis — severe bone loss of the primary dentition in a child diagnosed with AIDS.

odontitis, which includes mechanical therapy, antibiotic coverage, and maintenance.

Juvenile periodontitis also presents in either localized or generalized forms (17). Generalized juvenile periodontitis (GJP) usually occurs in the late teenage years and affects most teeth. The disease has been associated with a variable microbial etiology that may include *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* (24–26). Contributing risk factors such as smoking should be considered. Localized juvenile periodontitis (LJP) has an age of onset at or around puberty and is associated with molar and incisor bone and attachment loss (Figs. 3 and 4). However, atypical patterns of disease have been observed. The localized form is frequently characterized by association with the periodontal pathogen *Actinobacillus actinomycetemcomitans* and neutrophil function abnormalities (27). Both GJP and LJP may exhibit abnormalities in host immune cell functions that appear to follow a familial pattern.

The goal of treatment in early-onset periodontitis is to alter or eliminate the microbial etiology and the contributing risk factors, and faster regeneration of the periodontal apparatus. Due to the complexity of this type of periodontal disease with respect to systemic factors, immune defects, and the microbial flora, control of the disease may not be possible in all instances. In such cases, a reasonable treatment objective is to slow the progression of the disease by administering the appropriate antibiotic regimen, and providing repeated microbiological testing and an intensified, supportive periodontal therapy program.

Rapidly progressive periodontitis (RPP) is typically found in patients 20–35 years old. With the exception of the age of onset, the clinical, microbiological, and immunologic diagnostic findings in RPP are similar to those in GJP (17).

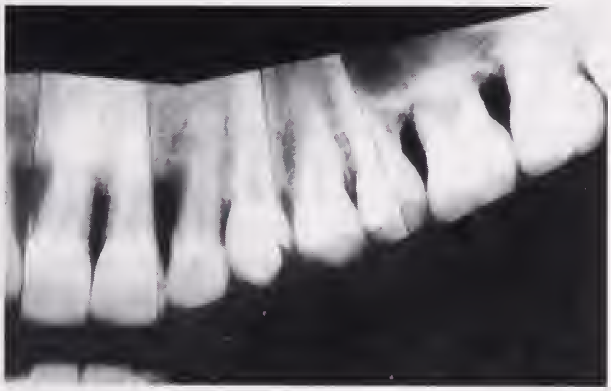

Fig. 3. Juvenile periodontitis — typical bone loss associated with the central incisors and first molars.

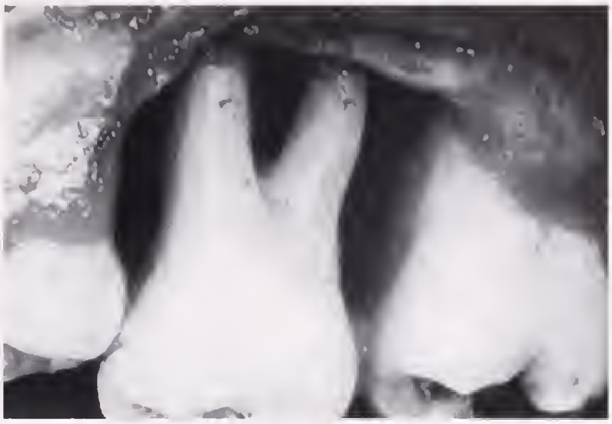

Fig. 4. Juvenile periodontitis — surgical exposure exemplifying the severe bone loss associated with the first molar.

The goals of periodontal therapy are similar to those in the treatment of adult periodontitis, including attempting the regeneration of the periodontal attachment apparatus where indicated. Due to the complexity of the early-onset periodontal diseases with regard to systemic factors, immune defects, and the microbial flora, control of the disease may not be possible in all instances. In such cases, a reasonable treatment objective is to slow the progression of the disease.

In general, treatment methods for early-onset periodontal diseases may be similar to those used for adult periodontitis. These methods should include oral hygiene instruction, and reinforcement and evaluation of the patient's plaque control; supra- and sub-gingival scaling and root planing to remove microbial plaque and calculus; control of other local factors; occlusal therapy as necessary; periodontal surgery as necessary; and supportive periodontal therapy. In addition, a general medical evaluation may determine if systemic disease is present in children and young adults who exhibit severe periodontitis, particularly if early-onset periodontitis appears to be resistant to therapy (28). Consultation with the patient's physician may be indicated to coordinate medical care in conjunction with periodontal therapy. In the early stages of disease, lesions may be treated with adjunctive antimicrobial therapy combined with scaling and root planing with or without surgical therapy. Microbiological identification and antibiotic sensitivity testing may be considered. The long-term outcome may depend upon the patient's compliance, and delivery of supportive periodontal therapy at appropriate intervals, as determined by the clinician. If primary teeth are affected, eruption of permanent teeth should be monitored to detect possible attachment loss.

Periodontitis Associated with Systemic Conditions

A number of systemic factors have been documented as capable of affecting the periodontium and/or treatment of periodontal disease. Systemic etiologic components may be suspected in patients who exhibit periodontal inflammation or destruction which appears disproportionate to the local irritants. Periodontal therapy may be modified based on the current medical status of the patients. Periodontal organisms may be the source of infections elsewhere in the body. Therefore, those infections may also affect systemic health.

The therapeutic goal is to achieve a degree of periodontal health consistent with the patient's overall health status. Achieving this goal, however, may be directly affected by the degree of control of the systemic condition. The systemic and psychological status of the patient should be identified, therefore, to reduce medical risks that may compromise or alter the periodontal treatment.

Patients with systemic conditions that contribute to progression of periodontal diseases may be successfully treated using established periodontal treatment techniques. However, the systemic/psychologic status of the periodontal patient may alter the nature of therapy rendered and may adversely affect treatment outcomes.

Metabolic Disorders

Diabetes Mellitus

Patients with undiagnosed or poorly controlled insulin dependent diabetes mellitus (IDDM) or non-insulin dependent diabetes mellitus (NIDDM) are at risk for periodontal disease. Periodontitis also progresses more rapidly in poorly controlled diabetics (29), and early age of onset of the disease is seen as a risk factor for more severe disease (30). Conversely, most well-controlled diabetic patients can maintain periodontal health and will respond favorably to periodontal therapy. Metabolic control is a prime factor in maintaining periodontal health among NIDDM patients (31 – 33), and there is some evidence for the converse, namely that periodontal health may affect diabetic control (34).

The suggested mechanisms by which diabetes may contribute to periodontitis include vascular changes, polymorphonuclear cell dysfunction, abnormal collagen synthesis, and genetic predisposition (35 – 37). While the mechanism by which diabetes exacerbates periodontal destruction is still not fully understood, periodontitis can be considered a complication of both types of diabetes.

Nearly all diabetics seem to respond to conventional periodontal therapy. Since diabetics are more prone to infections, adjunct prophylaxis may be necessary if surgical therapy is considered. To date, there are no reports of the specific use of antibiotics in diabetics and their effects on the outcome of therapy. More important, despite the modality of treatment chosen, the age of the patient, amount of plaque and calculus, and the level of diabetic control need to be taken into consideration.

Female Hormonal Alterations

Hormonal fluctuations in the female patient may alter the status of periodontal health (38, 39). Such changes may occur during puberty, the menstrual cycle, pregnancy, or menopause. Changes may also be associated with the use of oral contraceptives. The most pronounced periodontal changes occur during pregnancy, as a significant proportion of pregnant women suffer from pregnancy gingivitis (40). This transient and self-limiting condition includes an increase in bleeding, gingivitis, and a subgingival microbial shift. Gingival tissues return to their original healthy state postpartum when estrogen and progesterone levels reach baseline values. This reversal trend seems to parallel that observed in pubescent females. Aside from these transient changes, pregnant women in good health are unlikely to experience any significant gingival response that would have serious clinical implications. Women who are susceptible or have a pre-existing gingival condition should seek treatment to prevent extension of the inflammatory process, especially to avoid periodontal abscess formation that could cause a bacteremia. In general, pregnant women should note that preventive measures consisting of dental prophylaxis and meticulous plaque control help to prevent any periodontal condition from developing.

Women on hormonal replacement therapy and oral contraceptives experience a statistically significant increase in gingival inflammation (41, 42). With oral contraceptives, this increase in gingival inflammation is related to the duration of use, and results of recent studies suggest that prolonged use of oral contraceptives may detrimentally affect the periodontium (43).

In menopausal and/or postmenopausal women, osteoporosis is not considered an etiologic factor in periodontitis, though it may affect the severity of the disease in preexisting periodontitis (44). In general, women with preexisting gingival conditions or susceptibility to periodontal disease may experience an exacerbated response to bacterial plaque if they are pregnant, use oral contraceptives, have hormonal replacement therapy or undergo menopause. Given the effects of hormonal changes and their corresponding impact on the periodontium, proper periodontal evaluation and treatment for these women is recommended. For most healthy women without the predisposition to periodontal disease, the negative influence of the changes in estrogen and progesterone levels can be controlled by additional plaque control, and these women should not be considered to be at increased risk of periodontal disease.

Drug-Induced Disorders

Drugs can be a contributing etiologic factor in periodontal diseases. Drugs such as anticonvulsants, calcium channel blocking agents, and cyclosporine may induce gingival overgrowth (45) (Figs. 5 and 6). In addition, drugs can cause xerostomia, osteoporosis, lichenoid, and other hypersensitivity reactions. It is important, when possible, to have a baseline periodontal evaluation prior to initiation or modification of drug therapy. If there is gingival overgrowth, or other adverse reactions, there will be a need for modification of the drug regimen prescribed. Often surgery is necessary to eliminate gingival overgrowth, and this overgrowth may easily recur if drug therapy cannot be modified, and/or if there is not adequate plaque control on the part of the patient.

Hematologic Disorders / Leukemia

Hemorrhagic gingival overgrowth with or without necrosis is a common early manifestation of acute leukemia (46) (Fig. 7). Patients with chronic leukemia may experience similar but less severe periodontal changes. Chemotherapy or therapy associated with bone marrow transplantation may also adversely affect the gingiva (46, 47). Considerations for patients with hematologic disorders and periodontal disease should include coordination of treatment with the patient's physician. Prior to the treatment of leukemia and/or transplantation, appropriate periodontal therapy is rendered for minimization of sites of periodontal infection. If there is bone marrow transplantation, the patient should be monitored for evidence of host-versus-graft disease and drug-induced gingival overgrowth.

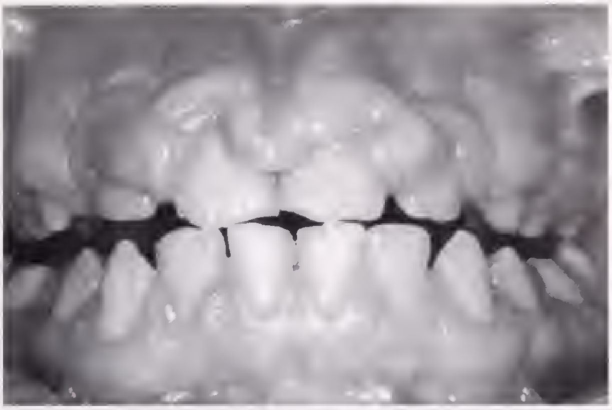

Fig. 5. Gingival overgrowth associated with the use of dilantin.

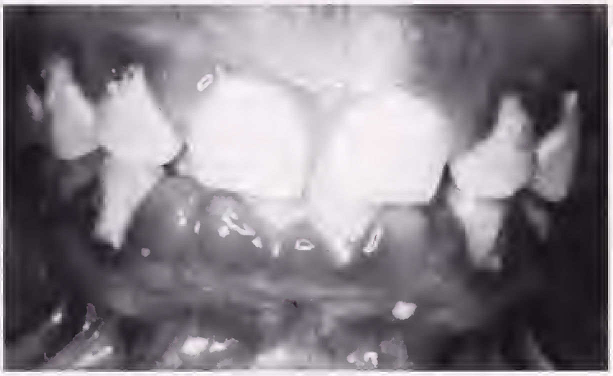

Fig. 6. Gingival overgrowth caused by cyclosporine in a patient with a kidney transplant.

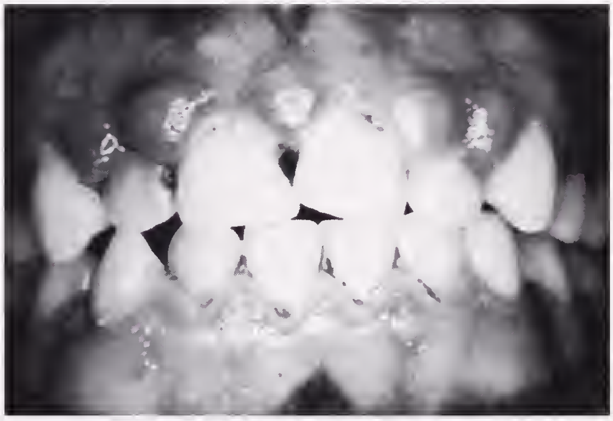

Fig. 7. Patient diagnosed with leukemia and associated hemorrhagic gingival overgrowth.

Immune System Disorders

Some forms of periodontal disease may be more severe in individuals with immune systems disorders. Patients infected with Human Immunodeficiency Virus (HIV), may have especially severe forms of periodontal disease, and this periodontal manifestation may often be the first clinical expression of the virus itself (48). The likelihood of acute necrotizing gingivitis and necrotizing periodontitis may increase in the patient with acquired immunodeficiency syndrome (AIDS) (49). Patients who have received organ transplants or are undergoing cancer treatment may be taking immunosuppressive medications. There are special considerations for immune system disorder patients with periodontal disease. Aside from being aware of the patient's immune status, the patient must be closely monitored for controlling associated mucosal diseases and acute periodontal infections.

The usual treatment involves gross scaling to remove visible plaque and calculus deposits, and the debridement of necrotic tissue when present. Following initial debridement, follow-up visits are necessary for close monitoring and evaluation of the oral tissues and the patient's plaque control, as well as removal of additional deposits. Home use of an antimicrobial mouth rinse such as chlorhexidine has been shown to be effective in reducing the acute symptoms and recurrence of lesions. Antibiotics should be used with caution, due to the increased risk of overgrowth of *Candida albicans* and other microflora associated with the HIV infection. The use of a concurrent antifungal agent has been recommended, as well as narrow spectrum antibiotics such as metronidazole, which leave the aerobic Gram positive flora undisturbed (50). The response to therapeutic intervention may, however, depend upon the patient's current immune status and/or HIV stage, intake of systemic medications, intake of antibiotics, and oral habits (e.g., tobacco smoking).

Conclusion

While many patients respond well to conventional periodontal therapy, others do not. Strategies that have improved our understanding of how bacteria cause medically important infections are starting to be successfully applied to examine mechanisms of bacterial pathogenesis in periodontitis. In order to rationally treat and prevent recurrence of periodontal diseases, we need to know the identity of etiologic agent(s) for specific patients, and the virulence properties of the pathogenic species involved. Future studies are necessary to determine these answers, and improve our understanding of the mechanism of bacterial pathogenesis in periodontitis. This knowledge, in turn, should lead to the development of highly specific antimicrobials to prevent and treat periodontal diseases.

What becomes evident from this review is that the periodontal tissues are often associated with manifestations of a wide range of systemic diseases. Early detection and carefully managed therapeutics with the physician and periodontist working hand-in-hand may prove beneficial to the patient's general health and quality of life. It is important that physicians recognize and familiarize themselves with these possible oral manifestations, and refer for the treatment of the periodontal disease. When there are signs of destructive oral disease of the hard and soft tissues, it is important that the dentist or periodontist determine if there may be an underlying systemic factor requiring referral to the physician.

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Oral Cancer and Oral Effects of Anticancer Therapy

Andrei Barasch, D.M.D., M.D.Sc.[1], Monika Safford, M.D.[2], and Ellen Eisenberg, D.M.D.[3]

Abstract

The term "oral cancer" generally refers to squamous cell carcinoma of oral mucosal origin, which accounts for more than 90% of all malignancies of this location. Although a relatively uncommon disease in the United States, this malignancy is nonetheless important, as it accounts for significant morbidity and mortality. Approximately half of the estimated 30 thousand cases diagnosed annually in this country have a fatal outcome. Survivors of the surgical and medical management may suffer from sequelae of treatment ranging from pain and infection to partial or total disfigurement and loss of stomatognathic function. Such high morbidity and mortality are truly regrettable, since many of these malignancies are preventable. This article will review recent developments in the epidemiology, treatment and chemoprevention of oral squamous cell carcinoma as they relate to early diagnosis, and management of the oral cancer patient. **Key Words:** Mouth, cancer, chemotherapy, etiology, epidemiology, radiation.

Epidemiology of Oral Cancer

Oral and pharyngeal cancers rank number five in prevalence worldwide, but represent only 3 – 5% of all malignant neoplasms diagnosed annually in the United States (1 – 3). The overall survival rate for this disease in 1987 was 50.7%, with incidence and mortality remaining virtually unchanged over the past two decades (1). This relatively high mortality is in part due to the advanced stage of the disease at the time of diagnosis. The 5-year survival rate for patients with localized disease at the time of diagnosis is approximately 75% as compared to less than 10% for those with distant metastases (1). Thus, early diagnosis of this easily identifiable malignancy, alone could drastically reduce mortality, medical costs, pain and suffering. Given the ready accessibility of the oral cavity, knowledgeable clinicians should be adept at recognizing precancerous abnormalities as well as frank carcinomas.

Despite a relatively constant incidence of oral cancer, national trends in the past 50 years show some significant epidemiological changes: the number of affected white males has steadily decreased, while a disturbing increase of this disease among African-American males and all females has been noted (1). In contrast, recent European studies report an alarming overall increased prevalence of oral cancer, particularly in younger adults from the Eastern and Central regions of that continent (4). However, this increase may be due partly to previous underreporting of cases before 1989.

While oral cancer remains predominantly a disease of males (in the United States, the male-to-female ratio was approximately 2.5 for the period 1983 – 1987) (1), a significant linear trend toward increasing prevalence of this malignancy in females has been reported, with ratios changing from 9.8:1 in 1935 to as low as 2.6:1 in 1985 (5).

From the Departments of [1]Dental Medicine and [2]Medicine, New Jersey Dental School, University of Medicine and Dentistry of New Jersey, Newark, NJ, and [3]Department of Oral Diagnosis, University of Connecticut School of Dental Medicine, Farmington, CT. [1]Assistant Professor; [2]Assistant Professor; [3]Professor and Director of Oral Pathology.

Address correspondence to Andrei Barasch, D.M.D., M.D.Sc., University of Medicine and Dentistry of New Jersey, New Jersey Dental School, Room C 829, 110 Bergen Street, Newark, NJ 07103-2400.

The number of women with oral cancer increased yearly by 0.5% despite the fact that the total number of reported lesions decreased by 0.4% within the analyzed 15-year period (1). This phenomenon can be explained in part by increased exposure to carcinogenic substances as a result of greater social acceptance of smoking and alcohol use in women. However, other factors may account, at least partially, for this observation, and they remain to be identified or confirmed (5, 6).

Most cases of oral cancer in the United States are diagnosed in the sixth and seventh decades of a person's life, with the highest prevalence noted in 65–74-year-old patients (1, 5). However, younger individuals, including children, may be affected in rare instances (7). In a study of more than 14,000 patients with oral squamous cell carcinoma, 3 were younger than 14 at the time of surgery and another 16 belonged to the 15–19-year-old group. Overall, 7.2% of the patients were younger than 40 (7). A similar report found 53 cases of tongue cancer in males 40 and younger, which represented a nonlinear increase in prevalence of the disease in this age group (8). These findings suggest a trend toward decreasing age at diagnosis of oral cancer. However, other reports do not support this conclusion, mandating further study of the issue (1, 5). Based on these suggestions and the European experience, examining younger patients with this diagnosis in mind is nevertheless advisable.

The majority of intraoral squamous cell carcinomas originate from the non-keratinized mucosa of the tongue and the floor of the mouth. Recently, a trend toward increased numbers of lesions arising on both the dentate and edentulous gingiva was reported, with the gingiva being the third most common site for intraoral squamous cell carcinoma in several studies (6, 9–13). These results are surprising, since keratinized tissues were traditionally considered to be relatively immune to factors contributing to malignant transformation in the oral cavity.

An explanation for these findings is not readily evident. However, two possible contributory factors can be listed: (a) etiologic agents other than tobacco and alcohol consumption may have more affinity for keratinized tissues; and (b) gingival carcinomas have a predilection for females, who account for an increasing number of oral cancer cases (6, 9). This theory is consistent with recent reports that suggest that alcohol and tobacco have unequal malignant transformation potential on specific oral subsites (floor of the mouth > tongue > gingiva) (6, 12–14). Similarly, gender may be a significant predictor of the oral subsite for development of cancer, with males and females showing predilection for development of floor of the mouth and gingival carcinoma, respectively (5, 6). However, due to lack of uniformity in study methodology in the published reports, solid conclusions regarding these topics are premature. Further study is necessary for confirmation of these findings, as well as for identification of possible mechanisms of carcinogenesis at various oral subsites.

Etiology of Oral Cancer

Many factors probably play a role in the development of oral cancer. Although there is debate with regard to the relative importance of some of these factors, it is widely agreed in Europe and North America that tobacco and alcohol play a significant role in the pathogenesis of carcinoma arising from oral surface epithelium (14, 15), whereas it is clear that ultraviolet radiation is the major cause of cancer of the external lip (16). Betel (areca) nut and toombak chewing are strongly associated with oral cancer in Southeast Asia and Africa (17). Other proposed causative agents include alcohol-containing mouthwashes, syphilis, poor oral hygiene, iron deficiency, chronic trauma and viruses; however, well-designed studies failed to show any consistent association of these factors with malignant transformation.

Another controversial topic in oral cancer etiology is the role of smokeless tobacco. While most authors strongly believe that chewing or pouching tobacco increases chances for oral epithelial malignant transformation (16, 18, 19), recent epidemiological data cast significant doubt on this association (20–22). This doubt is augmented by the fact that tobacco products have not been shown to induce premalignant changes in animal models. Since neither the mechanism of oral carcinogenesis nor the precise role of tobacco and alcohol has been elucidated, and since only approximately 3% of heavy users of these substances ever develop oral cancer, the search for other possible etiologic factors must continue. Recent efforts have been directed toward identification of a possible role of viral agents, particularly the human papilloma and herpes simplex viruses (HPV and HSV) (23, 24). These ubiquitous viruses have been associated with various other cancers and are common inhabitants of the oral cavity. While some studies found an increased presence of viral genome in malignant and premalignant oral lesions, others denied any conclusive evidence (23).

It is worth mentioning that evidence gathering for identification of cellular and molecular mechanisms of oral carcinogenesis is hampered by the relatively small number of cases in the United States, the large number of possible etiologies, and the lack of an adequate animal model. At this time, it appears safe to conclude that the etiology of oral squamous cell carcinoma is multifactorial and that various oral sites may be selectively affected by these factors.

Clinical Presentation

Premalignant oral lesions typically present as asymptomatic erythroplakia or leuko-erythroplakia of the non-keratinized soft tissues (25–27) (Fig. 1). Any such lesion without obvious cause, such as mechanical or chemical irritation, particularly if found in an elderly tobacco and/or alcohol user, should be regarded as suspicious. Submucous fibrosis is a premalignant presentation consisting of epithelial atrophy and induration with loss of elasticity of affected mucosa; it is associated with betel nut use. Patients are generally symptomatic, presenting with limited mouth opening, trismus and inability to eat.

Other clinical presentations of premalignant lesions are possible but uncommon. Leukoplakia, a white patch on the mucosa which cannot be otherwise diagnosed on clinical grounds alone, was thought to be the prototypical oral premalignant presentation in the past (28), but it has been shown to undergo malignant transformation in fewer than 4% of the cases (27). Another controversial lesion in terms of its malignant potential is lichen planus, which is often the subject of confusion with a similar clinical entity, lichenoid dysplasia (29). A recent genetic study provided more evidence that true lichen planus, an autoimmune disease, is most likely not premalignant (30). However, since the clinical presentations of lichen planus and lichenoid dysplasia are similar, a confirmation biopsy should be performed on all lichenoid-appearing lesions.

Clinical presentation of invasive malignancies in the oral cavity is typically in the form of a mass, nodule or an indurated ulcer (Figs. 2 and 3). Color changes are common and consist of red, or red and white hues. Attachment to adjacent tissues is possible, though not necessary, and represents local invasion of the tumor. Systemic spread occurs through the submandibular, cervical and jugular lymphatic pathways, and distant metastases most commonly occur in the lungs. Symptoms are uncommon in earlier stages of disease, but become frequent with advanced local invasion. In particular, paresthesia and anesthesia in the absence of a history of trauma are highly suggestive of invasive malignancy.

Since microscopy is the sole definitive means of distinguishing malignant tissues, biopsy is indi-

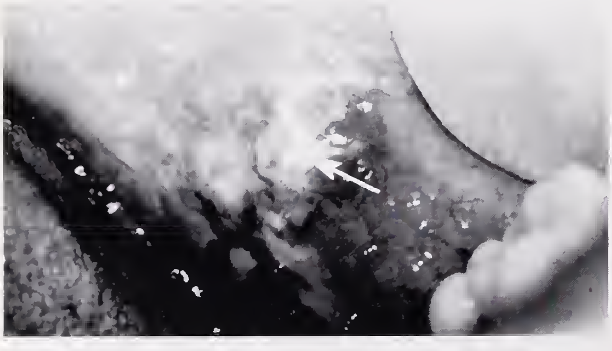

Fig. 1. Erythroleukoplakia of the buccal mucosa in a 74-year-old heavy-smoking female. This lesion was moderately dysplastic on microscopic examination.

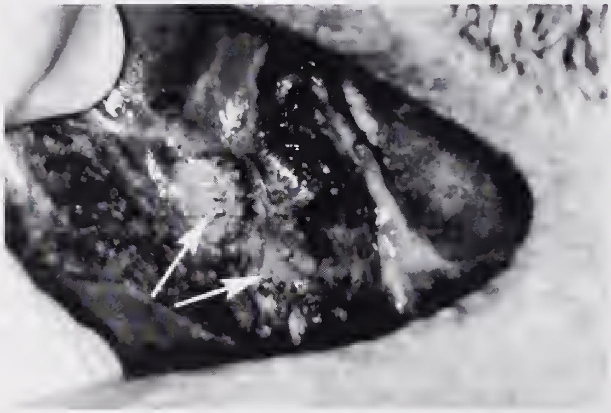

Fig. 2. Second primary squamous cell carcinoma on the buccal mucosa of a 91-year-old male.

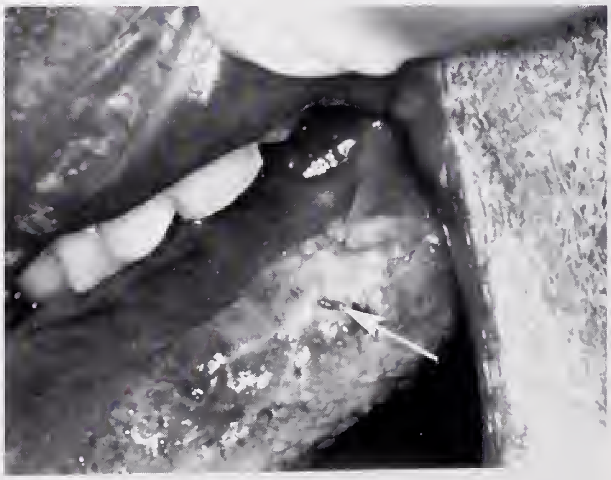

Fig. 3. Squamous cell carcinoma of the lateral tongue. An ideal biopsy should include the ulcerated area.

cated for all suspicious mucosal lesions. Although exfoliative cytology is an invaluable diagnostic tool for cervical malignancies of mucosal origin, it is not a reliable method for diagnosis of suspicious oral mucosal lesions (27). Once oral squamous cell carcinoma has been confirmed by biopsy, extensive upper aerodigestive mucosal examination is mandatory, since synchronous lesions occur in 10–20% of patients (31).

Therapeutic Approaches

Clinical staging of oral cancer is accomplished based on the tumor size, lymph node involvement and metastases (TNM) classification, which is further divided into Stages I–IV (32). Lymphatic and metastatic spread are best determined by computed tomography (CT) or magnetic resonance (MRI) studies followed by microscopic confirmation. Therapeutic strategies are usually dictated by the stage of the disease and the patient's ability and desire to withstand treatment. Similarly, prognosis is also dependent on the stage of the malignancy at initiation of therapy, the patient's compliance with the therapeutic regimen, and necessary lifestyle changes.

Stage I and II diseases have a high rate of complete remission, and 60–80% of the patients are disease-free 5 years after surgery or radiation therapy. Stage III and IV oral cancers have poor to dismal prognosis, and any curative attempts must include multitherapeutic approaches (33). Confirmed metastatic disease is typically treated palliatively. Most treatment failures consist of either local recurrence or a second primary tumor which carries a prognosis much worse than the initial disease. Continuation of tobacco and ethanol use, as well as the presence of genetic mutations in adjacent tissues (field cancerization), has been shown to significantly decrease chances for long-term survival.

The time-tested therapeutic approaches to oral cancer are surgery and ionizing radiation. Chemotherapy has been used in research trials as adjuvant or neoadjuvant therapy for the past two decades. While initial results were not encouraging, recent studies of concomitant or alternating chemo-radiation have reported significant therapeutic benefits for patients with advanced disease. These treatments have both benefits and distinct disadvantages.

Surgery is rapid and has few associated late side effects. However, due to the requirement of a 1.0 cm resection margin of normal tissue, it tends to be painful and mutilating, and results in various degrees of oral dysfunction. Compensatory approaches such as free or attached tissue flaps and prosthetic devices are often utilized to alleviate these problems. Additionally, the stress of the surgical procedure under general anesthesia and the lengthy healing process require that the patient be relatively healthy, with few other complicating medical problems.

Radiation therapy, unlike surgical resection, preserves the integrity of tissues with little or no immediate dysfunction. Inconveniences consist of the long duration (5–8 weeks) and the late effects of this therapeutic modality. Additionally, initial use of radiation therapy reduces or precludes the option of using this method for recurrences or second primaries in the same field. Typical radiation protocols consist of 54–72 Gy (Grays, a unit of absorbed radiation dose equal to 100 rads) of gamma irradiation delivered from an external source in 180–220 cGy/day (1 centiGray [cGy] = 1/100 Gy) for 5 days/week. During active radiation, patients experience various degrees of mucositis accompanied by possible dysphagia and/or odynophagia, and dry mouth sensation or xerostomia. Mucosal lesions can be severe enough to necessitate interruption of therapy and/or hospitalization with nutritional support, but typically heal without scarring 1–2 weeks after completion of treatment (Fig. 4). To date, attempts to prevent or reduce duration and severity of radiation-induced mucositis have produced little success. Thus, standard care for this painful condition consists of palliation of symptoms and prevention of infectious complications (34). Preliminary studies of benzydamine (an

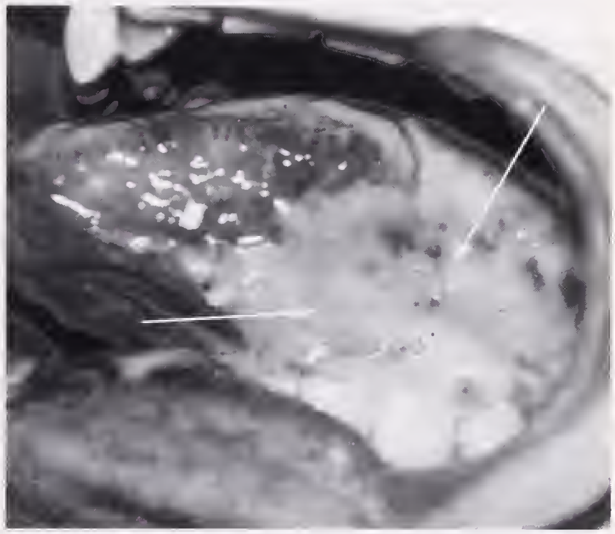

Fig. 4. Radiation-induced oral mucositis after 40 Gy of external beam radiation for floor of the mouth cancer.

anti-inflammatory medication), antimicrobial lozenges, and low-power laser therapy have shown some promise, with further investigation possibly establishing these modalities as efficacious prevention/therapy for this side effect (35, 36). Late effects of ionizing radiation typically appear weeks to months after completion of therapy and persist for the patient's lifetime. These effects result from tissue injury and fibrosis, with subsequent loss of function that is proportional to the amount of injury sustained by that tissue. In particular, salivary glands and intraosseous blood vessels can be affected irreversibly by this process, resulting in salivary hypofunction and risk for osteoradionecrosis, respectively (37).

Decrease in salivary production is not always correlated with the degree of xerostomia. However, salivary hypofunction does result in a proportionally increased risk for mucosal and dental infections, and patients are typically plagued by fungal overgrowth and dental caries (Fig. 5). Meticulous oral hygiene, augmented by chlorhexidine gluconate oral rinses and topically applied 1–2% fluoride gel, is mandatory for these patients (topical antifungal medications such as nystatin suspension and clotrimazole troches have a high sucrose concentration and are not recommended for dentate patients). Additionally, it is possible to increase salivary output in patients with residual gland function by using oral pilocarpine hydrochloride (Salagen®, MGI Pharma), at 5–10 mg three to four times daily (38, 39). Recent pilot studies suggested an advantage in salivary function preservation and patient comfort when pilocarpine is started during, rather than after, radiation therapy (40, 41). This advantage must be confirmed by larger, independent studies that will also assess effects of the regimen on tumor response and recurrence rate.

The definition of osteoradionecrosis (ORN) is still debated in the literature. Most experts in the field agree, however, that loss of bone vitality, with or without sequestration in the field of previous high dose ionizing radiation, is necessary to diagnose this condition (42). The pathogenesis of ORN consists of gradual fibrosis and narrowing of feeding intraosseous vessels, followed by infarction and tissue death (42, 43). The necrotic bone may be exposed to the oral environment spontaneously or by trauma to overlying mucosal tissues, and superinfection may follow with oral microorganisms (Fig. 6). This process correlates directly with the amount of radiation the bone has received and inversely with the supply of collateral circulation (44). The gradual deterioration of the local circulation is irreversible and reaches its peak approximately 6 months to a year after completing radiotherapy. Thus, all necessary oral surgical procedures should be completed either before or within 6 months after radiation (45). Patients requiring surgery after this period and those who develop spontaneous ORN can benefit from hyperbaric oxygen therapy (46, 47). Studies have shown this treatment to enhance wound healing and stimulate angiogenesis in irradiated tissues. The inconveniences of this treatment are its long duration (20–30 dives are recommended), its expense, and the paucity of available hyperbaric chambers. Recent case series reports have shown that successful surgical treatment in irradiated jaws as well as management of cases of ORN are possible even without the benefit of hyperbaric therapy (48, 49). It is still unclear, however, which cases can be managed conservatively and which stand to benefit most from the expensive hyperbaric chamber.

Use of cytotoxic medications alone for oral squamous cell carcinoma has shown no success in improving life expectancy in these patients. Despite a high percentage of initial partial and complete tumor response, a majority of these can-

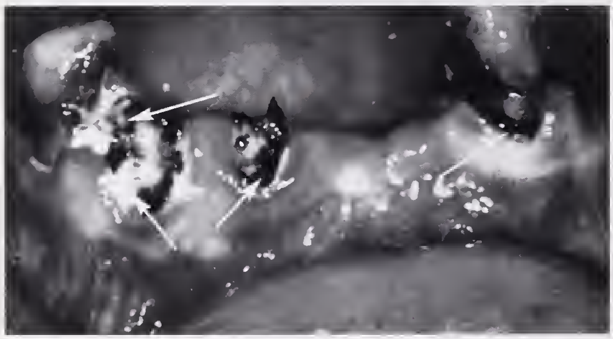

Fig. 5. Rampant caries 7 months status post 70 Gy radiation therapy.

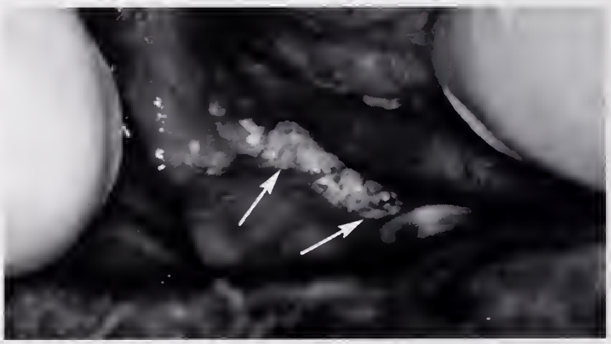

Fig. 6. Osteoradionecrosis of the mandible at 14 months status post 72 Gy radiation therapy. A tooth was extracted in that area one month prior to the photograph.

cers recur within months unless another therapeutic method is implemented (50). Neither adjuvant nor neoadjuvant protocols containing various drug combinations have demonstrated increased survival. Nevertheless, chemotherapy is the only systemic therapeutic approach available and thus represents the single current option for fighting metastatic disease. The high tumor response rate makes it plausible that cytotoxic drugs actually do eradicate microscopic disease (51), and this modality should be included in all cases where distant spread is suspected.

The standard of care for advanced oral and pharyngeal cancer consists of combined surgical and radiation therapy, most commonly in this chronological order. When surgery up-front is not feasible, radiation therapy may be followed by salvage surgery for elimination of residual disease. Recent attention has focused on treatment of advanced oral cancer with concomitant or alternating combination chemo-radiotherapy, and results have been encouraging thus far (52–54). These protocols have generally been applied to patients with stage III and IV disease. Most studies have shown the combination therapies to be superior in both survival and disease-free survival rates at the expense of more severe side effects during treatment. Finally, a recent study has resurrected the idea of neoadjuvant chemotherapy by reporting excellent results with a protocol of two rapid cycles of cytotoxic medications followed immediately by irradiation (55). Dose-limiting side effects of combination regimens are mainly local, with severe mucositis reported in more than 90% of the patients. This finding adds urgency to the quest for an effective mucositis-preventive protocol which may provide patient comfort and allow for more aggressive treatment, with better disease control and survival.

Cancer Chemoprevention

When discussing this topic, we must mention from the start that oral cancer chemoprevention research has been hampered by lack of a clear precancerous lesion and absence of biomarkers to measure intermediate outcomes. Thus, results published must be interpreted cautiously. The problem has two aspects: prevention of malignant transformation in committed premalignant tissues, and return to normal growth control patterns; and prevention of multiple primary tumors in tissues adjacent to a successfully treated carcinoma. The basic idea is that the carcinogenic process may be inhibited or reversed by administration of various exogenous compounds. Based on earlier evidence that malignant transformation favors patients deficient in certain nutrients, attention has focused on antioxidant substances from the retinoid (vitamin A) and tocopherol (vitamin E) families (56). Consistent and encouraging results were obtained in vitro and in animal studies. Several human trials, however, reported either no significant activity or unacceptable severity of side effects and recurrence of premalignant lesions after cessation of therapy (56, 57). Additionally, the Beta-Carotene and Retinol Efficacy Trial (CARET) has found that patients randomized to vitamin A and beta-carotene had an increased overall mortality and lung-cancer-related deaths (58). These studies are hard to reconcile with data showing increased risk for cancer in patients deficient in retinoids and other antioxidant substances. Once again, a better understanding of the malignant molecular process may elucidate these issues.

Conclusion

Cancer of the oral cavity and pharynx is still taking a high toll in those populations at risk. These lesions are easily visualized, and early detection could dramatically contribute to decreasing morbidity and mortality from this disease. Recent research has identified changes in epidemiological patterns and etiologic agents related to various oral subsites of oropharyngeal squamous cell carcinoma. Treatment of advanced stages of the disease is also changing to include the addition of chemotherapy to surgical and/or radiation protocols. Finally, contrary to expectations, chemopreventive regimens have been disappointing in their ability to control or reverse the malignant process. New clinical trials must incorporate the effects of various substances on oral mucosal tissues, if further progress is to be made in our understanding of carcinogenesis.

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Transmission of Infectious Disease in the Dental Setting

Jagdev Heir, D.M.D.[1], and Vincent B. Ziccardi, M.D., D.D.S.[2]

Abstract

Patients in the industrialized world are at low risk of acquiring nosocomial infections; nevertheless, several aspects of care in the hospital or clinic can place patients at risk from infectious disease transmission. Paramount to the prevention of disease is the strict adherence to universal precautions for all patients.

Tuberculosis has again emerged as a potential epidemic capable of dramatic morbidity and mortality. The World Health Organization estimated that 22.6 million people were infected with HIV and that 50 million would be infected by the year 2000. The main types of viral hepatitis are hepatitis A through G. The chance of transmitting hepatitis B virus is higher than the chance of transmitting HIV.

As knowledge regarding the pathophysiology and transmission of these diseases has increased in recent years, some insight has also been gained into the problems related to transmission of diseases between individuals. This paper will discuss the transmission of tuberculosis, hepatitis and HIV as related to the dental setting and health-care workers. **Key Words:** Tuberculosis, hepatitis, human immunodeficiency virus.

Introduction

Patients in the industrialized world are at low risk of acquiring nosocomial infections; nevertheless, several aspects of care in the hospital or clinic can place patients at risk from infectious disease transmission. Tuberculosis (TB), Human Immunodeficiency Virus (HIV), and hepatitis have long been considered work hazards for health-care workers (1–3). As knowledge regarding the pathophysiology and transmission of these diseases has increased in recent years, some insight has also been gained into the problems related to transmission of diseases from patient to patient, patient to health-care worker, and health-care worker to patient. This paper will discuss the transmission of TB, hepatitis and HIV as related to the dental setting and health-care workers. It is not intended to be a comprehensive review of disease transmission, but rather an overview as it relates to the clinical practice of dentistry.

Tuberculosis

Tuberculosis has again emerged as a potential epidemic capable of dramatic morbidity and mortality (4). From 1953, when the United States Center for Disease Control and Prevention (CDC) started monitoring TB, until 1984, the number of new cases of TB declined each year. However, recently there has been a reversal in this trend (5). There are several reasons why TB is on the rise: increasing number of homeless; crowded social living conditions in the inner cities; increasing numbers of immunocompromised patients with HIV, organ transplantation, and bone marrow transplantation; incorrect prescribing of drug regimens; and patient noncompliance with drug therapy for TB (6–9).

Tuberculosis is caused by *Mycobacterium tuberculosis, M. bovis, or M. africanum.* The

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From the Department of Oral and Maxillofacial Surgery, New Jersey Dental School, University of Medicine and Dentistry of New Jersey, Newark, NJ. [1]Junior resident and third year medical student and [2]Assistant Professor and Associate Residency Director.

Address all correspondence to Vincent B. Ziccardi, M.D., D.D.S., University of Medicine and Dentistry of New Jersey, New Jersey Dental School, Department of Oral and Maxillofacial Surgery, 110 Bergen Street, Room B 854, Newark, NJ 07103-2400.

mycobacteria are slender, curved, obligate aerobic, nonmotile rods (10). TB is spread via airborne particles which are aerosolized when an infected patient coughs, sneezes or speaks. These particles can remain in air circulation for extended periods of time. A person can get infected when these particles are inhaled into the lung. In the majority of cases, the immune system will ward off infection; however, 5 – 10% of individuals will develop active disease (11, 12). The development of infection is facilitated by increased concentration of infectious droplets in the air, inadequate ventilation, and re-circulation of infected air (13). High speed dental instrumentation creates an aerosol of water, saliva and potentially infectious droplets through the air/water irrigation systems necessary to prevent pulpal overheating during dental preparation. For this reason, it is appropriate to always have eye/face protection and adequate suction when using high speed rotary instrumentation.

The diagnosis of TB is made by a thorough history, physical examination, tuberculin skin test (purified protein derivative, PPD), chest radiograph, and the culture and identification of *Mycobacterium tuberculosis*. Diagnosis can take several weeks. Thus, certain methods must be utilized to decrease the incidence of infection. The appropriate approach to the dental patient who is suspected of being infected with TB is to use universal precautions, as is done with all patients. In this scenario, it is appropriate to perform emergent and palliative procedures; however, routine dentistry should be deferred until the patient is on appropriate drug therapy and the infective period has lapsed (Table 1). Once sputum cultures are negative, the patient is considered non-infective and it is appropriate to do routine dental therapy. Thus far, only one documented case has been reported of a dental provider contracting TB from a patient (14).

TABLE 1
Dental Management of the TB Patient

TB Status	Dental Management
Active TB (AFB+ sputum)	Emergency treatment until negative sputum.
Positive skin test with no clinical symptoms	Emergency treatment until disease verified
Past history of TB with unknown treatment	Emergency treatment until medical confirmation of disease status
Past history of TB with completed drug therapy	Treat as normal
Present history of TB with drug therapy compliance and three negative sputum cultures	Treat as normal

References 3, 10, 29 – 31.

Human Immunodeficiency Virus

The HIV virus is a human retrovirus which depends upon the enzyme reverse transcriptase (RNA directed DNA polymerase) to replicate itself within the host cells. HIV has a short latency period, after which time clinical symptoms become evident, with eventual progression to Acquired Immunodeficiency Syndrome (AIDS). In 1996, the World Health Organization estimated that 22.6 million people were infected with HIV and that 50 million would be infected by the year 2000 (15). HIV is transmitted via direct absorption (by needle puncture, or break in the skin or mucous membrane) of body fluids, including blood, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, saliva, as well as unfixed tissues, and human organs (16).

Symptomatic HIV infection results from the virus attacking the T4 (CD4) antigen and chemokine receptors (CCR5 and CXCR4), which serve as receptors for the virus. Normally, the CD4 cell is a helper-inducer lymphocyte which directs many other cells within the immune network. By definition, patients with positive HIV serology and CD4 count below 200 cells/µL are considered to have AIDS (17).

Compared to other health-care providers, dental professionals have a lower risk of being infected with HIV, due to the decreased amount of viral load in saliva which contains anti-HIV activity (17). Pure saliva does not contain viruses unless contaminated by blood. The salivary protective protein binds to the HIV gp120 envelope protein, rendering it non-infective or less infective. It has been estimated that the risk of transmission from patient to health-care provider is higher than transmission from health-care provider to patient (18). The CDC estimates that the risk of a health-care worker acquiring HIV from a patient after being stuck is 1 per 250 – 300 procedures, whereas the risk of a patient acquiring HIV from a health-care provider is 2.4 – 24 per 1,100,000 procedures when exposed by direct contact with body fluids via iatrogenic percutaneous exposure (16). Factors which increase the risk for HIV transmission include: deep intramuscular injury, donor source from a patient with terminal AIDS, injury with a sharp device with visible blood, and injury with hollow bore needles.

The hollow bore needle has a higher transmission of disease since it contains an inoculum of blood and virus.

There are 5 documented cases involving seroconversion from a health-care worker to patients after treatment by an HIV-infected dentist in Florida. The cause of this transmission is unknown, with cross-contamination of instruments being ruled out. In one study, not one of more than 15,000 patients who were treated by 32 infected health-care workers, including 16 dentists, has acquired HIV through routine operative care (19).

Once an exposure does occur, the exposed health-care worker should be given the option for chemoprophylaxis (20). The evaluation of the exposed individual should include possible anti-retroviral drug resistance of the HIV-infected source (20). Once informed consent is obtained, baseline antibody testing should be performed for both the patient and health-care worker with repeat testing at 6 weeks, 12 weeks, and 6 months post-exposure. Chemoprophylaxis should be initiated within the first hour after exposure if possible and continued for a total of 4 weeks. The chemotherapeutics offered are zidovudine (ZDV), which is an anti-retroviral agent, lamivudine (3TC), which is a nucleoside analog reverse transcriptase inhibitor, and indinavir (IDV), which is a protease inhibitor (21) (Table 2). (See reference for further information regarding chemotherapeutic protocol.)

Hepatitis

Exposed health care workers are at risk for the main types of viral hepatitis which are hepatitis A, B, C, D, E, F, and G. Herpes simplex, cytomegalovirus (CMV), and Epstein-Barr virus (EBV) may also cause hepatitis (22). The chance of transmitting hepatitis B virus (HBV) is about 30% per encounter, whereas the transmission rate of HIV with known contaminated blood is 0.3% (16). The risk is directly related to the extent of the contact with the contaminated body fluids.

TABLE 2
CDC recommendations for chemoprophylaxis after exposure to HIV

Source	Prophylaxis	Treatment
Large volume of blood (Deep injury with large hollow bore needle)	Recommend	Four weeks (ZDV, 3TC, IDV)
Fluid containing blood, semen, vaginal secretions, CSF, synovial, pleural, peritoneal, pericardial, and amniotic fluid.	Offer	Four weeks (ZDV, 3TC)
Urine	Not recommended	

ZDV = Zidovudine
3TC = Lamivudine
IDV = Indinavir

References 20, 21.

HBV is a DNA virus consisting of a DNA core, a DNA polymerase, and a core antigen contained in a protein shell. HBV has an incubation period of 30 – 120 days and clinically may cause jaundice, myalgia, arthralgia, and dermatological changes. A carrier state develops in 5 – 10% of people infected with hepatitis. Transmission usually occurs via blood and blood products in volumes of even less than 0.0001 ml (23). Every year, 12,000 health-care workers contract hepatitis B, and approximately 250 die of liver-related diseases. Mortality is usually about 1% in acute HBV infection in the general population, and is higher in patients infected with the delta agent and/or HIV. Immunization against hepatitis B is acquired via recombinant and yeast-derived vaccinations, which are 95% effective. Poor responders to vaccinations are patients on hemodialysis, immunosuppressed patients, the elderly and obese patients, smokers, and those with HIV-related disease. All health-care workers should be immunized against hepatitis B; however, this does not mean that they are protected from other hepatitides.

Hepatitis C (HCV), formerly known as non-A non-B hepatitis, is most commonly transmitted through parenteral exposure. HCV is an RNA virus that has 6 different genotypes located in different parts of the world. It accounts for 150,000 of all new cases of viral hepatitis in the United States (24). It may be transmitted via blood, blood products, IV drug abuse, immunoglobulin therapy, organ donations and, rarely, via sexual intercourse (25, 26). HCV has an incubation period of 25 – 90 days, with jaundice developing in 25% of patients. The virus may be detected in the saliva of as many as 50% of patients with acute and chronic hepatitis C infection (27). Treatment for HCV includes a-interferon, acyclovir, and ribavirin (23).

Hepatitis D (HDV) is an RNA virus that requires the presence of hepatitis B surface antigen for its transmission. HDV is spread via IV drug abuse, sexual contacts, blood or blood products. The incubation period is 28 – 140 days, with occurrence of jaundice and the development of cirrhosis in as many as 70% of infected patients. HDV is treated with interferon therapy and vaccination against HBV, which will indirectly protect against HDV.

Capilouto (28) studied the risk of becoming infected with HBV or HIV and found that through routine treatment of patients whose seropositivity is unknown, an annual cumulative risk of infection from HBV is 57 times greater than from HIV. In addition, the risk of dying from HBV infection is 1.7 times greater than that from HIV infection. This study has helped push health-care workers to undergo hepatitis vaccination. For those individuals who are exposed to HBV and are not immunized, immunoglobulin therapy and HBV vaccine series should be initiated immediately (Table 3).

TABLE 3
Differences between Hepatitis B, C, and D

	B	C	D
Onset	insidious	acute	unspecific
Latency	30–120 days	25–90 days	28–140 days
Jaundice	20%	25%	25%
Transmission	parenteral/ sexual	parenteral/ sexual	parenteral
Carrier	10%	100%	50%
Mortality	2%	30%	2%

Reference 32.

Conclusion

This paper has attempted to review the transmission of infectious disease in the health-care setting, specifically the dental operatory. Paramount to the prevention of disease is the strict adherence to universal precautions for all patients. This includes, but is not limited to, eye protection with lateral shields, face mask, and protective clothing which will be laundered on the premises or by appropriate services. Every medical center has available hotlines for needlesticks, with personnel specifically dedicated to that purpose. It is appropriate for health-care practitioners to familiarize themselves with these numbers and protocols in the event that they or ancillary staff are involved in an accidental exposure. Cooperation with the treating internist is critical to the safe and appropriate delivery of dental care to these patients.

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Diagnosis of Oral Ulcers

Lawrence C. Schneider, B.D.S., Ph.D.[1], and Adina E. Schneider, M.D.[2]

Abstract

Ulcers commonly occur in the mouth. Their causes range from minor irritation to malignancies and systemic diseases. Innocent solitary ulcerations, which result from trauma and infections, must be distinguished from squamous cell carcinomas, which also typically present as solitary ulcers. Multiple oral ulcers may be classified as acute, recurrent and/or chronic. The most common causes of rapid-onset oral ulcers include acute necrotizing ulcerative gingivitis, allergies and erythema multiforme. The two common forms of acute (short-term) recurrent oral ulcers, "cold sores" or "fever blisters," which are caused by the herpes simplex virus, and recurrent aphthous ulcers ("canker sores"), may be distinguished largely on the basis of their location. Most types of multiple chronic oral ulcers are associated with disturbances of the immune system. They include erosive lichen planus, mucous membrane pemphigoid and pemphigus vulgaris. Clinical criteria which are most useful in identifying the cause of oral ulcers are vesicles or bullae, which may not be seen because they rupture rapidly in the oral environment; constitutional signs and symptoms; and lesions on the skin and/or other mucosa. In some cases, diagnosis depends upon culture or biopsy, particularly with the application of immunofluorescence to the surgical specimen. **Key Words:** Oral ulcers, vesiculobullous oral lesions, gingival ulcers, mucosal ulcerations.

Diagnosis of Oral Ulcers

Ulcers occur in the mouth with considerable frequency. Traumatic lesions usually resolve rapidly and are not seen by clinicians. For relatively common conditions such as recurrent herpetic vesiculo-ulcers and aphthous ulcers, presumptive diagnoses are often made without recourse to laboratory tests. While the diagnosis of some types of oral ulcers is facilitated by their association with constitutional signs and symptoms or lesions on the skin and/or other mucous membranes, ulcers which are localized to the oral cavity may be more difficult to identify (Table). Several diseases are vesiculobullous; this feature is useful in narrowing the differential diagnosis, but lesions rupture so rapidly in the oral environment that the vesicular aspect of the ulcers is easily missed. There are, therefore, a wide variety of oral ulcers which are biopsied because they are less readily recognizable on the basis of their clinical features.

Most traumatic oral ulcers can be identified by their association with an identifiable mechanical, chemical, thermal or radiotherapeutic cause. They may be single or multiple, symmetrical or irregular in shape, and are usually painful. Most are of recent onset, but some are chronic. Acute traumatic ulcers have a removable, yellow-white base and erythematous borders. Chronic traumatic ulcers may be non-painful with an indurated base and raised borders; consequently, they may be indistinguishable from squamous cell carcinomas on the basis of their clinical features.

Multiple painful oral ulcers often occur as a result of radiotherapy for head and neck cancer and as a side effect of chemotherapy for many malignancies. Radiation mucositis often appears after the first week of treatment and focal ulcerations may develop within affected areas. The

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From the [1]Department of Oral Pathology, New Jersey Dental School, University of Medicine and Dentistry of New Jersey, Newark, NJ, and [2]Department of Medicine, Columbia University College of Physicians and Surgeons, New York, NY. [1]Professor and [2]Clinical Instructor.

Address correspondence to Lawrence C. Schneider, B.D.S., Ph.D., University of Medicine and Dentistry of New Jersey, New Jersey Dental School, Department of Oral Pathology, 110 Bergen Street, Newark, NJ 07103.

TABLE
Oral Ulcers

Acute	Multiple Ulcers, Recurrent	Chronic	Solitary Ulcers
Acute necrotizing ulcerative gingivitis	Aphthae	Allergies*	Aphthae
Allergies*	Herpes simplex virus, secondary*	Bullous pemphigoid*	Chancre
Chemotherapy		Epidermolysis bullosa*	Fungi (deep)
Erythema multiforme*		Lichen planus*	Gumma
Herpangina*		Lupus erythematosus	Necrotizing sialometaplasia
Herpes simplex virus, primary*		Mucous membrane pemphigoid*	Squamous cell carcinoma
Herpes zoster virus*		Paraneoplastic pemphigus*	Trauma
Mucous patches		Pemphigus vulgaris*	Tuberculosis
Radiotherapy			

* Vesicles or bullae may occur in these conditions

The table categorizes oral ulcers according to their number and chronology. Those are two features which are useful in their recognition.

mucositis persists for several weeks beyond the end of therapy and the mucosa remains atrophic. Anticancer drugs cause multiple oral ulcers by bone marrow suppression or by reducing replication of the oral epithelium. Both types of ulcers heal within a few weeks following the end of therapy.

Two forms of acute (short-term) recurrent oral ulcers are extremely common. Recurrent herpetic ulcers ("cold sores" or "fever blisters") may be distinguished from recurrent aphthous ulcers ("canker sores") primarily on the basis of their location. The recurrent lesions caused by the type I herpes simplex virus occur as a cluster of small vesicles which ulcerate on the vermilion of the lips or on adjacent skin (1). Each vesiculo-ulcer is only 1 mm or less in diameter. Recurrent herpetic lesions are uncommon inside the mouth in otherwise healthy people and occur as a cluster on keratinized mucosa which is bound to underlying bone, on the hard palate near the teeth or on the gingiva. Recurrent aphthous ulcers occur on areas of the mouth where the mucosa is non-keratinized and loosely attached, particularly the buccal mucosa, labial mucosa, floor of the mouth, ventral surface of the tongue, and soft palate. In contrast to the viral lesions, aphthous ulcers do not have a vesicular stage, and they are larger than the individual viral ulcers.

Recurrent aphthous ulcers (2) may affect 20% of the population. Immunologic, hereditary, and nutritional pathogenetic factors have their advocates, but none of the numerous theories is widely accepted. The ulcers may be single or multiple and are very painful. Minor aphthae are less than 1 cm in size, and symmetrical in shape, with a yellow base surrounded by a red halo. They heal spontaneously in less than 2 weeks, with ulcer-free periods varying from individual to individual. Major aphthae are much less common. They are larger, deeper, irregular in shape, may persist for several weeks, and may thus be mistaken for a malignant lesion. Major aphthae often heal with scarring, which distinguishes them from minor aphthae. Numerous forms of treatment have been recommended but topical corticosteroids still appear to be the most effective.

In most cases, recurrent aphthous ulcers are limited to the mouth, without a definitive correlation with any other disease. The lesions are occasionally the result of a systemic condition. Deficiencies of iron, folate and vitamin B-12 have been linked to the ulcers. Lesions resembling aphthae also occur in patients suffering from chronic inflammatory bowel disease, cyclic neutropenia, Behçet's syndrome and Reiter's syndrome. Particularly severe aphthous-type ulcers occur in HIV-positive individuals.

While the multiple acute vesiculo-ulcers in recurrent herpes simplex virus infection are limited to the lips and, rarely, to the keratinized areas of the oral cavity, a more widespread distribution of multiple acute vesiculo-ulcers occurs in primary herpetic gingivostomatitis, other viral infections and allergic conditions. Primary herpetic gingivostomatitis usually occurs in children who lack previous exposure and immunity to the virus. Vesicles develop throughout the mouth and perioral skin. Marked gingival involvement, in the form of edema and erythema, is a major feature of the disease. Constitutional manifestations are common, with the disease subsiding within two weeks, but localized recurrences, particularly herpes labialis, may occur. Although diagnosis is usually made clinically, it can be confirmed by various techniques. Viral isolation with immunotyping of the isolates is the most sensitive diagnostic test for herpes simplex virus, but results cannot be obtained for 2–4 days. Cytological smears obtained from a recently opened vesicle reveal acantholytic epithelial cells, some of which may be multinucleate and exhibit nuclear viral inclusions. This test is rapid but does not distinguish between the herpes simplex and zoster viruses. HSV-1 may be identified in a few hours if cytological specimens are subjected to direct immunofluorescence using kits which are commercially available (3). Serology for immunoglobulins is positive in primary herpes simplex infections in 1 week, reaching a peak in 3 weeks. A fourfold or greater elevation of circulating serum HSV-1 antibody levels in the convalescent sera is considered diagnostic of recent HSV-1 infection (4). Acyclovir is more effective in treating immunocompromised patients than people who are otherwise healthy.

Oral vesiculo-ulcers occur in varicella. Unilateral, clustered vesicles characterize herpes zoster. Although lesions are similar in appearance to those caused by the herpes simplex virus, they may be recognized by their distribution pattern and severe pain along the affected branch of the trigeminal nerve.

Herpangina is caused by the *Coxsackie* virus and is clinically manifested by vesicles limited to the pharynx and posterior region of the mouth. This disease occurs in epidemics, most often affecting children, usually in summer. Constitutional symptoms are mild, with resolution within 10 days.

Acute hypersensitivity reactions to systemically administered drugs or foods include erythema, vesicles and ulcers of the oral mucosa, which are often associated with concurrent skin lesions. Similar oral lesions occur as a result of contact allergies but are less common. Cinnamon is a well-documented example of an offending agent.

Erythema multiforme (5) is an ulcerative mucocutaneous disease, some cases of which appear to be an acute hypersensitivity reaction to systemically administered drugs, to foods, or to the herpes simplex virus. Intact vesicles or bullae rupture rapidly. Although there is widespread mucosal involvement with severe crusting of the lips, the gingiva are characteristically spared. The oral condition is usually accompanied by skin lesions. The combination of oral, ocular and genital lesions is known as Stevens-Johnson syndrome.

Erythema multiforme is sometimes confused with primary herpetic gingivostomatitis. Both are vesiculo-ulcerative conditions with sudden onset, most often affecting young people. They may be distinguished, however, by the shape and distribution pattern of the oral lesions. Herpetic ulcers are small, round and shallow, and are accompanied by gingival edema, whereas the lesions of erythema multiforme are larger and more irregular in shape. Erythema multiforme is also characterized by severe ulceration and crusting of the lips, with little gingival involvement. That diagnosis is, of course, much more obvious if typical skin lesions are present.

Acute ulcerative necrotizing gingivitis ("trench mouth") is another ulcerative disease of sudden onset. It primarily affects the gingiva, but other parts of the mouth may also be involved. Gingiva are bright red and hemorrhagic. Painful non-vesicular ulcerations first appear on the interdental papillae (the small triangular areas of mucosa between the crowns of adjacent teeth). The ulcers extend along the marginal gingiva and are covered by a gray necrotic pseudomembrane. Fever, malaise, and lymphadenopathy may be present. Anaerobic organisms are involved. The number of the fusospirochetal organisms correlates with the course of the disease, but impaired local and systemic host resistance is of major importance in this condition. Local treatment consists of debridement and irrigation. Antibiotics may be required.

Rapidly progressive periodontal disease with soft tissue necrosis and exposure of underlying bone occurs in HIV patients (6). The condition is characterized by deep, aching pain in the jaws and a lack of response to conventional treatment. In severely debilitated individuals, acute ulcerative necrotizing gingivitis can spread to other parts of the mouth by direct extension or by contact with adjacent mucosa. In the condition known as necrotizing stomatitis, noma or cancrum oris, extensive soft tissue loss and bone sequestration can occur with large areas of discolored necrotic tissue (7).

Mucous patches are the most common oral manifestation of secondary syphilis. The ulcerations are irregular in outline, covered by a gray-white necrotic membrane and surrounded by erythema. They may be multiple and are sometimes painful. "Snail-track" ulcers are confluent mucous patches which heal in a few weeks. Less common oral lesions include roseolas, which are small red-brown macules, papules and condyloma lata. If these oral lesions, constitutional symptoms, and the typical macular-papular skin rash are absent, mucous patches may be confused with other types of multiple oral ulcers.

Squamous cell carcinoma is one condition which presents as a solitary, chronic ulcer of the oral mucosa. While advanced lesions are readily recognized by their cratered appearance, with an indurated base and rolled borders, early lesions may easily be missed. Small, painless indurations, erosions, or red macules which fail to heal when any possible source of irritation is removed should be considered to be malignant until proven otherwise. This approach is particularly valid when there is a history of smoking and alcohol consumption, and when lesions occur in the high risk sites, which are the lateral and ventral surfaces of the tongue, the floor of the mouth, and the soft palate. Incisional biopsy is the only dependable way to distinguish squamous cell carcinoma from benign chronic solitary ulcerations, which include traumatic lesions, major aphthous ulcers, and infectious ulcers.

Necrotizing sialometaplasia (8) is a dramatic and benign condition which may be mistaken both clinically and histologically for a malignancy. Most cases occur unilaterally on the posterior hard palate, but the lesions may be bilateral or may occur on the soft palate and in other parts of the mouth. It most often presents as a deep ulceration of up to several centimeters in diameter and has irregular, well-defined borders which are neither indurated nor raised. The ulcer base is uneven and yellow or gray in color, and may be painful. Patients report that the lesion had developed very rapidly (sometimes in a few days) and that the roofs of their mouths suddenly fell out, with spontaneous healing occurring in a period of several weeks to months. However, only those clinicians with the highest level of intestinal fortitude rely on its clinical features for their diagnosis. Pathologists who are familiar with the condition have no difficulty making the diagnosis on an incisional biopsy specimen.

Multiple chronic oral vesiculo-ulcers occur in mucous membrane pemphigoid, pemphigus vulgaris, paraneoplastic pemphigus, epidermolysis bullosa and occasionally in lichen planus. Mucous membrane pemphigoid (5, 9) is an auto-immune disease which most often occurs in women over the age of forty. Lesions are often hemorrhagic and may heal with scarring. They are often Nikolsky positive: a superficial layer of apparently healthy mucosa detaches from underlying tissue when gentle pressure or an air-jet is applied. Other mucous membranes may be affected. Scarring may occur, hence the synonymous term "cicatricial pemphigoid." Conjunctival involvement may result in blindness due to scarring. Mucous membrane pemphigoid is a major cause of desquamative gingivitis, which is described below. Cleavage occurs in the subepithelial zone. In the subtypes of mucous membrane pemphigoid, autoantibodies are produced against various antigens in this region, including BPAG1, an intracellular, hemidesmosomal desmoplakin, BPAG2, a transmembrane protein in the basal plasmalemma of basal keratinocytes, and epiligrin (laminin 5), a matrix protein in the lamina lucida of the basal lamina.

Diagnosis of some multiple-ulcerative conditions, including mucous membrane pemphigoid, depends upon recognition of the tissue level at which clefting and/or necrosis occurs and the application of the direct immunofluorescent technique (10). The biopsy specimen should include perilesional (clinically normal) tissue. (Some laboratories request two biopsies, one from the edge of a fresh lesion and the other from normal-appearing mucosa, at least 3 mm from the lesion.) The specimen is divided into halves. One piece is placed in 10% formalin for routine staining with hematoxylin and eosin. If the other cannot be frozen immediately, it is placed in Michel's solution until direct immunofluorescence can be performed. Because of the expense, this is not carried out routinely. The specimen is incubated with fluorescein-labeled antibody against the autoantibody (which is bound to antigen in the tissue). The bound fluorescein emits a bright yellow-green light when the tissue is exposed to ultraviolet light.

Multiple oral vesiculo-ulcers occur less commonly in several other auto-immune, subepithelial cleavage diseases, including bullous pemphigoid, linear IgA bullous dermatoses, dermatitis herpetiformis and epidermolysis bullosa acquisita, each of which is characterized by different antigens (10). Severe oral vesiculo-ulcers also occur in some of the inherited (non-autoimmune) types of epidermolysis bullosa, particularly the recessive, dystrophic form (11).

Pemphigus vulgaris (5, 9) is an autoimmune, mucocutaneous, potentially fatal disease in which the oral mucosa is usually affected. Cleavage occurs within the stratified squamous epithelium due to autoantibodies against the desmosomal component, desmoglein 3. In the mouth, thin-walled vesicles and bullae rupture quickly and the lesions continue to increase in size, producing large, irregular, shallow ulcerations. It may be distinguished from other chronic, vesiculo-ulcerative diseases by the identifying rounded, acantholytic epithelial cells in exfoliative cytology specimens obtained by opening an intact vesicle, but biopsy is more reliable for definitive diagnosis. Direct immunofluorescence is usually unnecessary for diagnosis, but indirect immunofluorescence, which detects circulating antibodies, is valuable in monitoring progress of the disease while it is treated with steroids. Paraneoplastic pemphigus (12) is an extremely severe form of pemphigus which occurs in association with malignancies, particularly non-Hodgkin's lymphomas and certain leukemias. Cleavage occurs both within the epithelium and in the basement membrane zone. There are circulating autoantibodies against both desmosomal and hemidesmosomal antigens.

Lichen planus (13) is a chronic autoimmune condition which occurs in middle age, slightly more often in women than in men. Oral lesions may occur in the absence of skin lesions. They tend to have a bilateral and symmetrical distribution in the mouth. In the erosive form, the ulcers cause a burning sensation and may be accompanied by more typical, white reticular lesions. There are rare cases in which bullae are seen. Cleavage is due to destruction of basal epithelial cells associated with an infiltration of T-lymphocytes into this layer. It is one of the more common causes of desquamative gingivitis. Treatment is steroid administration, preferably topical.

Desquamative gingivitis (14) is the term applied to red, edematous, and glazed gingiva. There may be areas of superficial ulceration or desquamation which may exhibit Nikolsky's sign. This gingival condition is seen in mucous membrane pemphigoid, erosive lichen planus, pemphigus vulgaris, drug and food reactions, as well as epidermolysis bullosa acquisita, linear IgA dermatosis, and systemic lupus erythematosus. Some cases affect only the gingiva, and in some of these no antigens or antibodies are detected.

Conclusion

The various types of oral ulcers may appear clinically to be very similar. Features which are helpful in identifying the cause of ulcers are the associated constitutional signs and symptoms, presence of lesions on the skin and/or other mucosa, and the presence of bullae and vesicles. In some cases, however, laboratory procedures are required to make the diagnosis. Biopsy is necessary in the management of several of these conditions, especially in multiple ulcers due to autoimmune diseases, necessitating the application of immunofluorescence to the surgical specimen for definitive diagnosis.

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Antibiotic Prophylaxis in Dentistry

André Montazem, D.M.D., M.D.

Abstract

Antibiotic prophylaxis for the prevention of infective endocarditis related to dental therapy is a widespread therapeutic intervention. Recently, updated and revised guidelines on this treatment modality have been set forth in the form of consensus statements. It is evident that the risk of spontaneous bacteremia is much greater in a diseased oral cavity than it is in a healthy oral cavity. Perhaps most important in the prevention of infective endocarditis is early identification of at-risk patients and prompt referral to oral health specialists for comprehensive evaluation and treatment. The judicious use of antibiotic prophylaxis for prevention of infective endocarditis, in conjunction with optimal oral health care, should serve to minimize the adverse effects of antibiotic therapy and at the same time reduce the significant morbidity and mortality associated with these infections. **Key Words:** Antibiotic prophylaxis, infective endocarditis, prosthesis, dentistry.

One of the most common indications for the use of antibiotic prophylaxis in dentistry has been for the prevention of infective endocarditis and infections resulting from implanted prosthetic devices or materials. The topic of antibiotic prophylaxis in dentistry has been the subject of considerable controversy and debate. Numerous studies on the use and misuse of antibiotic prophylaxis have been performed (1 – 5). Cost-benefit analysis is likely to become the main determinant of its continued use. For this reason, the rationale, as well as the appropriate indications, for its use must be determined. Recently, two major conjoint panels have convened to apprise the health care community on these very issues (6, 7).

The cost-benefit ratio of any therapy, including all potential adverse effects, must be determined. Studies of this nature with respect to the treatment of infective endocarditis have already been conducted (8 – 15). The injudicious use of antibiotic therapy has proven to be expensive as well as directly responsible for development of resistant microorganisms.

The most common adverse effects of antibiotic therapy are listed in Table 1. Certainly, many of the adverse effects are of minimal clinical significance and have little impact. On an acute basis, anaphylaxis is the most feared of the possible consequences, and the risk of this potentially fatal complication has been studied (16 – 18). Of greater concern over the long term is the progressive development of drug-resistant organisms (19, 20). This is a potentially catastrophic problem which is difficult to assess. In addition, the financial implications of developing new drug therapies and treating patients with multi-drug-resistant infections are certain to be enormous. Therefore, it is incumbent upon the health care community to determine which patients would benefit most clearly from prophylactic antibiotic therapy and those who would not. The adverse outcomes associated with use of antibiotic therapy must be assessed in comparison to the costs and morbidity related to treating infective endocarditis or infected prosthetic materials. If the risk-benefit and cost-benefit ratios are critically examined, it becomes clear that if there are specific therapeutic indications based on sound physiologic, anatomic and scientific evidence, then

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From the Department of Oral and Maxillofacial Surgery, Mount Sinai School of Medicine, New York, NY.

Address correspondence to André Montazem, D.M.D., M.D. Assistant Clinical Professor, Department of Oral and Maxillofacial Surgery, Mount Sinai School of Medicine, One East 100th Street, Box 1187, New York, NY 10029.

antibiotic prophylactic therapy may be justified.

The risk of complications with therapy (Table 1) must be assessed, stratified and compared with complications when antibiotic therapy is withheld. When these risks have been elucidated, appropriate recommendations can then be made. The stratification of risks must be based on the likelihood of developing the disease and the probable outcome should the patient develop disease (1, 21–23). These factors could be classified as risk categories based upon the morbidity and mortality associated with the outcome. The American Dental Association (ADA) in conjunction with the American Heart Association (AHA) convened a panel to provide updated information on the following: risk stratification and classification based upon a review of the current literature; pharmacology; pharmacokinetics; and microbiology associated with the oral cavity. The risk stratification scheme, as developed by the panel of experts, is seen in Table 2.

The most common microbial flora of the oral cavity, as well as the most common oral flora implicated in infective endocarditis, have been identified (Table 3) (24, 25). With the aid of the recently convened panel, updated information has been provided on those procedures most likely to result in transient bacteremias which may seed anatomic irregularities and prosthetic materials. In addition, the most effective therapies for these organisms have also been clearly defined (Table 4) (26). In brief, the use of antibiotic therapy must aim to cover the normal flora of the oral cavity, namely streptococcal species. Further, an appropriate antibiotic must be administered in therapeutic doses prior to those procedures that are likely to result in bacteremia (Table 5).

TABLE 1
Complications of Antibiotic Therapy

Direct Toxicity
- Gastrointestinal
  - (e.g,. nausea, vomiting, diarrhea, abdominal pain)
- Hematologic
  - (e.g., neutropenia, thrombocytopenia, hemolysis)
- Alteration in normal flora
  - (e.g., pseudomembranous colitis, candidiasis)
- Nephrotoxicity
  - (e.g., renal function test, abnormalities, proteinuria, renal failure)
- Neuropathy
  - (e.g., VIIIth nerve dysfunction, peripheral neuropathy)
- Drug interactions
  - (e.g., clarithromycin-terfenadine, altered clearance)
- Hepatobiliary
  - (e.g., jaundice, liver function test, abnormalities, hepatitis)

Hypersensitivity Reactions
- Cutaneous eruptions
  - (e.g., rash, urticaria, exfoliative dermatitis)
- Serum sickness (e.g., immune complex reactions)
- Immediate hypersensitivity
  - (e.g., penicillin anaphylaxis, bronchospasm, laryngeal edema)

Development of Antibiotic Resistance
- Short- and long-term

TABLE 2
Risk Stratification for Infective Endocarditis

High Risk Category
- Prosthetic cardiac valves, including bioprosthetic and homograft valves
- Previous bacterial endocarditis
- Complex cyanotic congenital heart disease
  - (e.g., single ventricle states, transposition of the great arteries, tetralogy of Fallot)
- Surgically constructed systemic pulmonary shunts or conduits

Moderate Risk Category
- Most other congenital cardiac malformations
  - (other than above or below)
- Acquired valvar dysfunction
  - (e.g., rheumatic heart disease)
- Hypertrophic cardiomyopathy
- Mitral valve prolapse with valvar regurgitation and/or thickened leaflets

Endocarditis Prophylaxis Not Recommended
- Negligible-risk category
  - (no greater risk than the general population)
- Isolated secundum atrial septal defect
- Surgical repair of the atrial septal defect, ventricular septal defect, or patent ductus arteriosus
  - (without residua beyond 6 months)
- Previous coronary artery bypass graft surgery
- Mitral valve prolapse without valvular regurgitation
- Physiologic, functional, or innocent heart murmurs
- Previous Kawasaki disease without valvular dysfunction
- Previous rheumatic fever without valvular dysfunction
- Cardiac pacemakers (intravascular and epicardial) and implanted defibrillators

From Dajani AS, Taubert KA, Wilson W, et al. Prevention of bacterial endocarditis. Recommendations by the American Heart Association. JAMA 1997; 277:1794–1801 (6).

TABLE 3
Microbiology of Endocarditis

Streptococci	50.3%
Staphylococci	24.9%
Enterococci	6.1%
Gram-negative	5.7%
Fungi	1.0%
Other	2.7%
Culture negative	9.3%

Adapted from Tunkel AR, Mandell GL. Infecting microorganisms. In: Kaye D. editor. Infective endocarditis. 2nd ed. New York: Raven Press; 1992. p. 86 (24).

The use of antibiotic therapy as prophylaxis for patients with prosthetic implants has been questioned. The joint panel representing the American Academy of Orthopedic Surgeons and the ADA has defined the appropriate therapeutic

TABLE 4
Pharmacotherapy for Prevention

Situation	Agent	Regimen[†]
Standard general prophylaxis	Amoxicillin	Adults–2.0 g; children–50 mg/kg orally 1 h before procedure.
Unable to take oral medication	Ampicillin	Adults–2.0 g IM or IV; children–50 mg/kg IM or IV within 30 min before procedure.
Allergic to penicillin	Clindamycin or	Adults–600 mg; children–20 mg/kg orally 1 h before procedure.
	Cephalexin[+] or cefadroxil[+]	Adults–2.0 g; children–50 mg/kg orally 1 h before procedure.
	Azithromycin or clarithromycin	Adults–500 mg; children–15mg/kg orally 1 h before procedure.
Allergic to penicillin and unable to take medications	Clindamycin or	Adults–600 mg; children–20 mg/kg IV within 30 min before procedure.
	Cefazolin[+]	Adults–1.0 g; children–25mg/kg IM or IV within 30 min before procedure.
High-risk patients	Ampicillin plus gentamicin	Adults–ampicillin 2.0 g IM or IV (not to exceed 2.0 g) plus gentamicin 1.5 mg/kg (not to exceed 120 mg) within 30 min before procedure; 6 h later, ampicillin 1 g IM/IV or amoxicillin 1 g orally.
		Children–ampicillin 50 mg/kg IM or IV (not to exceed 2.0 g) plus gentamicin 1.5 mg/kg within 30 min before procedure; 6 h later, ampicillin, 25 mg/kg IM/IV or amoxicillin 25 mg/kg orally.
High-risk patients allergic to ampicillin/amoxicillin	Vancomycin plus gentamicin	Adults–vancomycin 1.0 g IV over 1–2 h plus gentamicin 1.5 mg/kg IV/IM (not to exceed 120 mg); complete injection/infusion within 30 min before procedure.
		Children–vancomycin 20 mg/kg IV over 1–2 h plus gentamicin 1.5 mg/kg IV/IM; complete injection/infusion within 30 min before procedure.
Moderate-risk patients	Amoxicillin or ampicillin	Adults–amoxicillin 2.0 g orally 1 h before procedure, or ampicillin 2.0 g IV/IM within 30 min before procedure.
		Children–amoxicillin 50 mg/kg orally 1 h before procedure or ampicillin 50 mg/kg IM/IV within 30 min before procedure.
Moderate-risk patients allergic to ampicillin/amoxicillin	Vancomycin	Adults–vancomycin 1.0 g IV over 1–2h; complete infusion within 30 min before procedure.
		Children–vancomycin 20 mg/kg IV over 1–2 h; complete infusion within 30 min before procedure.

IM= intramuscular; IV= intravenous.
*Total children's dose should not exceed adult dose.
[+] Cephalosporins should not be used in individuals with immediate-type hypersensitivity reaction (urticaria, angioedema, or anaphylaxis) to penicillin.
[†] No second dose of vancomycin or gentamicin is recommended

From Dajani AS, Taubert KA, Wilson W, et al. Prevention of bacterial endocarditis. Recommendations by the American Heart Association. JAMA 1997; 277:1794–1801 (6).

TABLE 5
Dental Procedure and Endocarditis Prophylaxis

Endocarditis Prophylaxis Recommended*
- Dental extractions
- Periodontal procedure including surgery, scaling and root planing, probing, and recall maintenance
- Dental implant placement
- Endodontic (root canal) instrumentation or surgery only beyond the apex
- Subgingival placement of antibiotic fibers or strips
- Initial placement of orthodontic bands but not brackets
- Intraligamentary local anesthetic injections
- Prophylactic cleaning of teeth or implants where bleeding is anticipated

Endocarditis Prophylaxis Not Recommended
- Restorative dentistry + (operative and prosthodontic) with or without retraction cord ++
- Local anesthetic injections (non-intraligamentary)
- Intracanal endodontic treatment; post placement and buildup placement of rubber dams
- Postoperative suture removal
- Placement of removable prosthodontic or orthodontic appliances
- Taking of oral impressions
- Fluoride treatments
- Taking of oral radiographs
- Orthodontic appliance adjustment
- Shedding of primary teeth

*Prophylaxis is recommended for patients with high- and moderate-risk cardiac conditions
+ This includes restoration of decayed teeth (filling cavities) and replacement of missing teeth.
++ Clinical judgement may indicate antibiotic use in selected circumstances that may create
significant bleeding

From Dajani AS, Taubert KA, Wilson W, et al. Prevention of bacterial endocarditis. Recommendations by the American Heart Association. JAMA 1997; 277:1794–1801 (6).

TABLE 6
Patients at Potential Increased Risk of Hematogenous Total Joint Infection

Immunocompromised/Immunosuppressed Patients
- Inflammatory arthropathies: rheumatoid arthritis, systemic lupus erythematosus
- Disease-, drug- or radiation-induced immunosuppression

Other Patients
- Insulin-dependent (Type 1) diabetes
- First 2 years following joint replacement
- Previous prosthetic joint infections
- Hemophilia

Advisory Statement. Antibiotic prophylaxis for dental patients with total joint replacements. American Dental Association; American Academy of Orthopaedic Surgeons. J Am Dent Assoc 1997; 128:1004–1008 (7).

indications for prophylaxis in patients with prosthetic implants; it is summarized in Table 6. The most significant factor appears to be the use of prosthetic materials and the time frame in which they have been placed. Studies have shown that the incidence of infection decreases over time and that, after a critical interval, the use of antibiotic prophylaxis can no longer be substantiated based on the currently available information (27). However, mitigating factors such as immunocompetence of the patient, when the implant was placed, and the type of implant placed still may justify their use but clearly not on a routine basis.

Special situations may arise which do not fit neatly into the currently recommended guidelines, and in these cases clinical judgment must be used in formulating the therapeutic plan. There may be certain factors in any particular case that may justify the use of prophylactic antibiotic therapy. These guidelines clearly denote that they are not intended to substitute for proper clinical judgment. However, any decision made must be based on the most current and accurate information available, as well as a thorough understanding of the pathophysiology, attendant risks of treatment and nontreatment, and the microbiology and pharmacology associated with the condition.

Finally, it is prudent to note that the likelihood of developing bacteremia and its related adverse outcomes is related to the health of the oral cavity (28–30). Patients who are likely to be at risk for infective endocarditis or who may require prosthetic grafts should always undergo dental evaluation. Appropriate preventive medicine and dentistry can greatly benefit those patients at risk. Referral to the dental specialist for comprehensive evaluation and treatment is indicated, because carious teeth and periodontal disease are contributory factors in bacteremic episodes (1). Proper oral health can minimize bad outcomes by eliminating sources of infection (31, 32). Until prior dental referral is included in the pre-operative evaluation, it is likely that significant numbers of patients will require antibiotic prophylaxis for urgent or emergent procedures. When patients undergo comprehensive evaluation, a formal treatment plan may be established. In this way, multiple procedures may be performed at the same visit, thereby reducing the number of appointments and the number of times that the patient will need prophylaxis. This would decrease costs, reduce adverse drug effects and lessen both the short- and long-term emergence of drug-resistant bacteria (33–35).

Summary

It has become apparent that a person with a diseased oral cavity is at high risk for developing spontaneous bacteremia after dental intervention. Because spontaneous bacteremia cannot be prevented, it behooves the health care team to man-

date dental evaluation and treatment for their patients who are at risk for complications related to bacteremia. Even in the well-cared-for mouth, the need for urgent care will arise; but the likelihood of this can be minimized with proper evaluation and care. The judicious use of antibiotic prophylaxis for prevention of infective endocarditis in conjunction with optimal oral health care should serve to minimize the adverse effects of antibiotic therapy and at the same time reduce the significant morbidity and mortality associated with these infections.

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The Impact of Language as a Barrier to Effective Health Care in an Underserved Urban Hispanic Community

RAND A. DAVID, M.D., AND MICHELLE RHEE, B.A.

Abstract

Background: Language barriers between patient and physician impact upon effective health care. This phenomenon is not well studied in the literature.

Methods: A survey was created in English and Spanish, and administered at the ambulatory site for medical housestaff and faculty at a teaching hospital. "Cases" were defined as patients who reported using a translator or as having poor English skills. Patients who reported not using a translator and having good English skills served as controls. Both groups were predominantly of Hispanic origin.

Results: Analysis revealed 68 cases and 193 controls. The survey completion rate was 96%. The data were predominantly categorical. Chi-square analysis was utilized. Both groups responded that understanding medication side effects corresponds to compliance (87% cases vs 93% controls, $p = 0.18$). More cases responded that side effects were not explained (47% vs 16%, $p < 0.001$). More controls reported satisfaction with medical care (93% vs 84%, $p < 0.05$). More controls agreed that their doctors understood how they were feeling, with statistical significance in Hispanic subset analysis (87% vs 72%, $p < 0.05$). Both groups felt they had enough time to communicate with their doctors (89% vs 88%, $p = 0.86$). More cases than controls reported having had a mammogram within the last 2 years (78% vs 60%, $p < 0.05$).

Conclusions: Lack of explanation of side effects to medication appeared to correlate negatively with compliance with medication. The language barrier correlated negatively with patient satisfaction. Cases reported more preventive testing; test ordering may replace dialogue.

Key Words: Doctor-patient relationship, Hispanic, language, non-English speaking, patient satisfaction.

Introduction

IT HAS BEEN DEMONSTRATED that the language barrier is an independent variable responsible for Spanish-speaking Hispanics having worse health status than English-speaking Hispanics (1). An analysis of 21 studies from the MEDLINE database, 1983–1993 (2), revealed that the quality of the physician-patient communication was positively correlated with improved health outcome. It has also been noted that, for various logistical and perhaps other reasons, research studies tend to exclude the non-English-speaking population (3).

In response to the above findings, we examined how a language barrier between patient and physician impacts upon selected measures which are representative of the health status of an individual. We focused on compliance with medications, patient satisfaction with medical care, and preventive testing.

Methods

Site and Subjects

The study site was the ambulatory primary care practice of the faculty and housestaff of a major teaching hospital. During the study period, 115 house staff and 20 full-time faculty of the Division of General Medicine attended the prac-

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From the Division of General Medicine, Department of Medicine, Mount Sinai School of Medicine, New York, NY.

Address correspondence to Rand A. David, M.D., Director, Ambulatory Care, Elmhurst Hospital Center, 79-01 Broadway, Room D1-24, Elmhurst, NY 11373.

Some of the results were presented as a poster for research day, Mount Sinai School of Medicine, Department of Medicine, on October 29, 1996. An oral presentation of the main results was given during medical student research day, Mount Sinai School of Medicine, on February 26, 1997. An abstract of the preliminary results was published (J Gen Intern Med April 1997; 12:Suppl 1:123) for the 20th national meeting of the Society of General Internal Medicine in Washington, D.C., May 1–3, 1997.

tice. The locale was inner city and designated as medically underserved.

Patients were drawn from the clinic population, which is comprised of individuals who are predominantly Spanish-speaking.

Medical office assistants were available to all patients to serve as translators. They aided in interpretation and gave explanations to patients, under the guidance of the physicians. However, they had had no formal training in the skill of translating.

Survey

An evaluative instrument was developed, in both English and Spanish, as a 5-minute survey. Patients were asked to respond by either "Yes" ("Sí") or "No" ("No") to a series of written questions regarding their medical care. First, they were questioned as to their age and gender. Then patients were requested to rate their Spanish and English verbal skills as excellent, good, fair, or poor. They were also asked whether or not they used a translator in order to communicate with their physician.

To evaluate the impact of patients' knowledge of the side effects of their medication on their compliance, we asked whether and from whom (physicians, nurses, pharmacists, medical assistants, others) they received information regarding medications. We further asked if receiving this information influenced their compliance with therapy.

We examined patient satisfaction for its value as an indicator of clinical outcome (4). We asked patients if they had enough time with their doctor, if their doctor understood how they were feeling, and if they were satisfied with their medical care.

For preventive testing, we selected mammography and cervical cancer screening. Each of these is included as a measure within the Health Plan Employer Data and Information Set 3.0 (HEDIS 3.0) (4), which is increasingly being implemented in the current managed care era. Beginning in January 1997, these measures became required for managed Medicare and Medicaid monitoring by the Health Care Financing Administration (HCFA) (4). In accord with the HEDIS 3.0 guidelines, female patients were asked whether they had had a mammogram in the previous 2 years and if they had had a PAP test in the previous 3 years.

Institutional review board (IRB) approval for the study was obtained, and the procurement of informed consent in writing was waived. The return of a completed survey was determined by the IRB to be indicative of implied consent.

Data Collection

Data collection occurred on days patients when visited their primary care providers. Over a 3-week period, consecutive patients were given the survey after registering for a visit to their primary physician. Patients were asked to complete the survey while waiting to see their primary physician. This created a cross-sectional view of clinic attendees.The practice site was composed of four geographic areas. The survey was given out in only one area of the practice at any particular time, in order to minimize interference with the flow of the practice. The area was randomly rotated with each successive day. On any given day, either a morning or afternoon session was alternatively surveyed, also to minimize disruption of the practice flow.

Data Analysis

According to the following predetermined criteria, the surveys were separated into cases and controls after they were returned:

1. Responders were defined as "cases" if they reported on the survey that they had used a translator in the past or had poor verbal English skills.
2. Responders were defined as "controls" if they reported that they had not used a translator and that they had fair-to-excellent verbal English skills.

A "Yes" or "Sí" response was considered a positive result in answer to questions regarding being given information about side effects to their medicines, whether they were satisfied with their medical care, and whether they had had mammography or Pap tests performed. A "No" was considered a negative response to these questions.

The data are predominantly categorical and a chi-square analysis was utilized. The exception to this is patient age, for which the Student's t test was utilized.

Further analysis of the subset of solely Hispanic cases and controls was carried out. This was done in order to examine whether language barrier impacted independently upon measured outcomes, while controlling for cultural barrier between patient and physician.

Results

The survey completion rate was 261 of 272 (96%). Eleven of 272 (4%) were not returned; these represented non-consenters and patients who had called to see their physician before they completed the survey. Cases were separated for analysis from controls in accordance with the above-predetermined criteria, based upon survey responses. A total of 68 cases and 193 controls were identified. Of the 68 cases, 4 reported having poor English skills but not having utilized a translator. This was too small a number to compare separately with cases who had used a translator, or with controls, who had not used a translator. Baseline characteristics are described in Table 1. Results for the entire population are described in Table 2. Subset analysis of Hispanic cases and controls is noted in Table 3.

TABLE 1

Baseline Characteristics of Population Studied

	Cases n = 68	Controls n = 193	p Value
Males	21%	22%	0.89
Females	80%	79%	0.89
Age	56 years 14–86	49 years 18–88	0.03
Hispanic	79%	44%	<0.001
African-American	12%	37%	<0.001
Caucasian	2.9%	9.3%	0.09
Asian	1.5%	3.6%	0.37
Other	4.4%	6.2%	0.59

TABLE 2

Summary of Results

	Cases n = 68	Controls n = 193	p Value
Understanding of Side Effects Corresponds to Compliance with Medication	87%	93%	0.18
Side Effects of Medication Were Explained	53%	84%	<0.001
Satisfaction with Medical Care	84%	94%	<0.05
Their Doctor Understands How They Feel	76%	85%	0.13
Feel They Have Enough Time to Communicate with Doctor	89%	88%	0.86
Doctor Discusses Mammography and Cervical Cancer Screening Tests	92%	88%	0.40
Have Had a Mammogram within the Last Two Years	78%	60%	<0.05

TABLE 3

Subset Of Hispanic Cases Vs Hispanic Controls

	Cases n = 54	Controls n = 85	p Value
Male	22%	20%	0.76
Female	78%	80%	0.76
Age	57 years 14–86	47 years 18–82	0.16
Understanding of Side Effects Corresponds to Compliance with Medication	88%	91%	0.64
Side Effects of Medication Were Explained	53%	88%	<0.001
Satisfaction with Medical Care	80%	95%	<0.05
Their Doctor Understands How They Feel	72%	88%	<0.05
Feel They Have Enough Time to Communicate with Doctor	88%	88%	1.00
Doctor Discusses Mammography and Cervical Cancer Screening Tests	90%	91%	0.84
Have Had a Mammogram within the Last Two Years	74%	58%	0.09

Compliance with Medication Regimen

Both cases and controls responded that an understanding of side effects corresponded to compliance with any proposed regimen (59 of 68 (87%) of cases vs 179 of 193 (93%) of controls, $p = 0.18$). Despite this similarity in the two groups, 53% of cases reported that side effects of medication were explained to them, in contrast to 84% of controls ($p < 0.001$), a statistically significant difference.

Patient Satisfaction

A higher percentage of controls reported satisfaction with medical care (181 of 193 = 94% of controls vs 57 of 68 = 84% of cases, $p < 0.05$). More controls agreed that their doctors understand how they felt, but the difference did not reach statistical significance for the whole population (52 of 68 (85%) of cases vs 163 of 193 (76%) of controls, $p = 0.13$); subset analysis of only Hispanics did in fact achieve statistical significance (39 of 54 (72%) of Hispanic cases vs 75 of 85 (88%) of Hispanic controls $p < 0.05$). Both cases and controls felt they had had enough time to communicate with their doctors (61 of 68 (89%) of cases vs 170 of 193 (88%) of controls, $p = 0.86$).

Preventive Testing (HEDIS-Related Measures)

Among the female patients, both cases and controls reported that their doctors had discussed mammography and cervical cancer screening tests at some point in time (50 of 54 [92%] of cases vs 132 of 150 [88%] of controls, $p = 0.40$). However, more cases than controls reported having had a mammogram within the previous two years (42 of 54 [78%] of cases vs 90 of 150 [60%] of controls, $p < 0.05$).

Discussion

Adherence to a medication regimen has been shown to be less likely when a patient does not clearly understand his or her physician's instructions (5, 6). Conversely, clear communication of medication instructions has been shown to be of critical value in adherence to a medication regimen (6). It was demonstrated that the simple practice of writing dosing information as number of times per day (e.g., q.i.d.) rather than hourly intervals (e.g., q6h) can significantly improve patient interpretation of instructions and compliance with therapy (6).

A language barrier is an obvious challenge to be overcome during a discussion regarding medications. We found that communication of side effects to prescribed medications is an additional issue in compliance with therapy. The lack of explanations regarding potential side effects was reported by patients to have a negative impact on their compliance with medication. We conclude that an intervention as basic as explanation of a medication's side effects is likely to increase compliance with therapy. Particular effort is needed when patients have multiple medical problems and are on complex regimens (5). These patients are more likely to lack a thorough understanding of their medication regimen (5). They are also more likely to have increased noncompliance leading to excess hospitalizations (7). We did not quantify the amount of polypharmacy in our study population. This is an important issue for us to pursue in our clinic practice.

In our study, the language barrier was reported to impact negatively on patient satisfaction. This may be related to our finding that patients for whom a language barrier exists are more likely to feel that their physician doesn't understand how they feel. Patients did report that amount of time spent with the physician was not a factor in their level of satisfaction with care. This is interesting, since the additional time required to utilize a translator might be expected to create time pressure for the physician. A priori, one might have contemplated whether this could have led to a reduction in the amount of time available to deliver effective care.

Interestingly, cases reported a higher percentage of preventive testing. This finding differs from a study performed utilizing another Hispanic population, Mexican-American women (8). In their study, Suarez and Pulley (8) found that English was a positive predictor of both recent Pap smear and mammography. The difference found may lie in the fact that our population was enrolled in an ongoing primary care practice. Perhaps referring patients for preventive testing served in part as a substitute for verbal communication in our practice. It seems plausible that test ordering is easier than dialogue.

One shortcoming of our study was the lack of documentation as to whether patients may have either over-reported or under-reported preventive testing. Our population has had a significant tendency to obtain services from multiple institutions, making this documentation impractical.

Since the control group was culturally very similar to the case group, it would be expected that any bias due to cultural barriers between patient and physician would apply fairly equally to both groups. The validity of our subset analysis rests on this assumption. Our results were statistically significant when we analyzed the subset of solely Hispanic cases compared to Hispanic controls. We therefore conclude that it is likely that the differences found between the groups were due to a language barrier. It is notable that cases were somewhat older than controls, although among the subset of solely Hispanic cases and Hispanic controls this was not statistically significant (Tables 1 and 3). Whether or not this is related to a tendency towards younger immigrants learning a second language is not apparent from our study. Another weakness of our study is that we did not undertake to ascertain educational background in the survey. It would be important to know if this would impact as a confounding or an independent variable for compliance with medical therapy, patient satisfaction, and preventive testing in our population.

The foundation of primary care is the physician-patient relationship. A great obstacle to the therapeutic bond occurs when a language barrier exists between the doctor and patient. More attention needs to be given to the process of language translation during this interaction. This could be achieved through several different approaches. Spanish and other language courses for physicians is one option. However, it has been demonstrated that brief language courses can lead to significant mistakes on the part of the provider regarding a patient's complaints (9). Specific focus on explanations of medications and their side effects with the help of existing translators may be helpful. Written information in the patient's native language is another option. Bilingual medical assistants, nurses, or pharmacists may also assist in conveying this information. Interventional clinical trials are necessary to define methods which could reduce the impact of language barriers on the delivery of effective health care.

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The Importance of the Family Context in Inflammatory Bowel Disease

Mary-Joan Gerson, Ph.D.[1], Jacqueline Schonholtz, M.A.[2],
Catherine H. Grega, Psy.D.[2], and Deborah R. Barr, Ph.D.[3]

Abstract

Background: This study examines psychological correlates of inflammatory bowel disease (IBD) to answer these questions: What is the association between individual and family stress levels and the patient's coping ability and severity of illness? How does the level of family functioning correlate with individual coping and illness severity? To what extent are patient and family beliefs about IBD related to symptomatology?

Methods: Seventeen patients and 44 of their family members were studied intensively in an exploration of the psychological response to IBD. They completed a comprehensive battery of self-report questionnaires assessing stressful life events, coping related to the illness, and beliefs regarding IBD. Ratings of family functioning were made from videotaped family discussions about IBD. Treating physicians made independent ratings of past and current disease severity.

Results: Surprisingly, recent disease activity was negatively correlated with family stress level ($r = -0.794$), but was not associated with patient stress level. However, impaired coping with IBD by affected individuals was directly correlated with both individual ($r = 0.595$) and family-wide ($r = 0.724$) reported stress levels. Disease severity was also found to be responsive to family communication style ($r = 0.431$) and to level of family agreement ($r = 0.531$) regarding the etiology and management of IBD.

Conclusions: These findings suggest that individual stress affects coping with IBD, whereas family-wide stress, communication style, and agreement about disease etiology and management are related to disease activity. The interrelationship of family and patient as well as individual coping with IBD should be evaluated in future studies.

Key Words: Crohn's disease, inflammatory bowel disease, ulcerative colitis, family, stress, coping.

Inflammatory bowel disease (IBD) is a chronic medical illness with acknowledged primary organic etiology whose psychological correlates have attracted interest for a long time. Originally, psychoanalytically derived hypotheses focused on the presence of a symbiotic relationship between the affected individual and his or her mother, and it has been proposed that unexpressed anger and disruptions in the mother-child relationship were associated with increased disease severity (1, 2). Associations between psychiatric diagnosis and IBD also have been proposed. After several decades in which inconsistent findings were reported in the literature, a meta-analysis of 138 studies involving patients with ulcerative colitis indicated that psychiatric diagnosis was not significantly correlated with disease status or severity (3). Moderately significant correlations between depression and Crohn's disease have been reported, but have not as yet been subjected to meta-analysis (4). It has also been proposed that stress can cause and exacerbate IBD. However, two recent prospective studies found no relationship between stressful life events and symptom exacerbation (5, 6).

We examined IBD from a "family systems" perspective, which views the patient in the context of his or her family environment. From this perspective, the disease process can be conceptualized as both systemic and circular. In the latter case, there is presumed to be a two-way causality between illness (denoting the experience of the

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From the Graduate Program in Clinical Psychology, New York University, and Division of Gastroenterology, Department of Medicine, Mount Sinai School of Medicine, as well as New School for Social Research, New York, NY. [1]Adjunct Clinical Professor of Psychology, New York University, and Adjunct Clinical Instructor, Mount Sinai School of Medicine, [2]graduate students at New York University, [3]graduate student at New School for Social Research. This study was supported by a grant from Martin Edelston of Boardroom, Inc.

Address correspondence to Mary-Joan Gerson, Ph.D., 80 Central Park West, Suite C, New York, NY 10023.

disease, including stress and coping [7, 8]) and symptomatology. Though circular processes have been introduced into the IBD literature, we significantly expanded this approach to include the familial relationships in which the patient and his or her disease are embedded. Because this represents a new, uncharted perspective, this study is necessarily exploratory in its methodology. Specifically, we explored the following questions: What is the association between individual and family stress levels, and the patient's coping ability and severity of illness? How does the level of family functioning correlate with coping and illness severity? To what extent are patient beliefs about IBD and family beliefs about IBD related to symptomatology?

Materials and Methods

Subjects

Seventeen subjects who had either ulcerative colitis (n = 7) or Crohn's disease (n = 10) and 44 of their family members participated in the study. All subjects were under the ongoing care of a gastroenterologist experienced in the treatment of IBD. All subjects were seen during the course of one calendar year. Diagnoses were confirmed using clinical history, as well as standard radiographic, endoscopic and histological criteria.

Individuals were presented with the opportunity to participate in the study by their treating gastroenterologists. Physicians reported that neither the degree of psychological health nor the physical condition of the patient influenced their discussion of the study with potential subjects. Of the 51% of patients who declined participation, almost all declined because of logistic and geographic inconveniences which were not influenced by socioeconomic or ethnic factors. Physicians completed the Inflammatory Bowel Disease Severity Index at the time of patient referral to the study.

Individuals willing to participate were contacted and interviewed in person no later than 2 months after physician referral. The 13 unmarried subjects were interviewed with their parents and siblings. There was a total of 15 siblings but no family had more than 2 siblings. The 4 married subjects were interviewed with their spouses.

Interviews were conducted either at the principal investigator's office or in the family's home. After separately completing a series of questionnaires, each participant was videotaped discussing IBD, focusing on issues raised by one of the questionnaires (Inflammatory Bowel Disease Severity Inventory [IBDI]; see Table 1). Upon departure, participants were given an additional packet of questionnaires to complete and mail to the senior author. All ratings and scoring of measures were done blind to physician evaluations of disease severity. All scoring and ratings in the study measures were performed by advanced clinical psychology students.

TABLE 1

Inflammatory Bowel Disease Inventory (IBDI)

1. What do you think ulcerative colitis (or Crohn's disease) is, actually?
2. In your opinion, what are the causes of ulcerative colitis (or Crohn's disease)? Please put a "1" next to the cause you think is most important, a "2" next to the next-most important cause, and so on.
3. List any precipitating events that may have led up to the onset of ulcerative colitis (or Crohn's disease) in your family.
4. If there have been any recurrent bouts of ulcerative colitis (or Crohn's disease), list possible precipitating events.
5. In what ways does ulcerative colitis (or Crohn's disease) present a problem for you in daily life? Please put a "1" next to the most important problem, a "2" next to the next-most important problem, and so on.
6. In what ways does ulcerative colitis (or Crohn's disease) present a problem for your family in daily life? Please number your items as you did for question 5.
7. How is ulcerative colitis (or Crohn's disease) treated in your family? What steps are taken to keep the disease under control and create good health?
8. Would you be likely to join a group of families who each have a member with ulcerative colitis (or Crohn's disease) in order to discuss common problems? Why or why not?

Measures

Multiple indices were used to measure individual and family variables. With the exception of the Inflammatory Bowel Disease Inventory (IBDI) and the Family Structure and Communication Index (FSCI), both of which were created for this study, all measures have been widely used in psychological investigations of disease coping.

Disease activity was scored using the Inflammatory Bowel Disease Severity Index, a physician summary rating of both recent and long-term severity of clinical disease, that utilizes an established clinical grading system (5). A rating of 1 ("very well") indicates remission with occasional medication for minor symptom flare-ups. A rating of 4 ("severe") indicates consideration for hospitalization, significant complications and chronic steroid therapy.

Stress was indexed using the Schedule of Recent Experience (SRE), a validated scale which measures the frequency and timing of stressful life events in the recent past (test-retest r = 0.78–0.83) (9), as well as the Family Inventory of Life Events and Changes (FILE), a validated measure of family-wide stressful life events (test-retest r = 0.64–0.84; Cronbach α = 0.81) (10). Two summary scores, which included chronic and recent stressors, were derived from the FILE: the Recent Life Change score and the Total FILE score.

Coping with IBD was assessed by the Experience Scale, established as a valid self-report scale for measuring psychological adjustment to living with IBD (Cronbach α = 0.84) (11), addresses such issues as the degree to which disease compromises mood, activity, and interpersonal relationships.

Beliefs regarding etiology and management of IBD were investigated using the Inflammatory Bowel Disease Inventory (IBDI), which is a self-report measure developed specifically for this study (Table 1). Inter-rater reliability (see **Subjects**), which was determined by dividing the number of categorizations where there was agreement between two raters by the total number of categorizations, was excellent (0.96). Additionally, the percentage of category agreement among family members was calculated, and a total family IBDI Agreement Score was computed by averaging the percentages of family agreement across questions. The degree to which beliefs were organized as blaming self or others was derived from the written IBDI responses, that is, the presence of explaining beliefs about etiology and management in terms of personal irresponsibility and neglect. Different raters evaluated the blaming dimension and the belief categorization. Inter-rater reliability for blame category was excellent (0.92) and was determined by dividing the the number of cases where there was agreement between two raters by the total number of cases.

Family relationship functioning was analyzed using the FSCI, a video-analysis of family relationship functioning developed specifically for this study, based on the videotaped family discussion of the 8 questions comprising the IBDI. Four theoretically basic aspects of family functioning (communication, boundaries, alliances, affect) are rated along a 7-point continuum. These are: communication (clarity, equality of participation); boundaries (rigid, invaded); alliances (flexible, dominant); and affect (open, varied). Interrater reliability, which was very good (0.84), was computed by dividing the number of agreed-upon ratings by the total number of ratings assigned.

Two other assessments were the Family Crisis-Oriented Personal Evaluation Scale (F-COPES), a well-validated self-report measure used to identify problem-solving approaches and behaviors used by families in response to problems or difficulties (test-retest; r = 0.81; Cronbach α = 0.77) (12), and the Family Environmental Scale (FES), a widely used, multi-dimensional self-report measure of the "family environment" (test-retest r = 0.68–0.86) (13). Scores from the interpersonal relationships dimension (FES-A) were computed only for this study.

Statistical Analyses

T-tests comparing subjects with ulcerative colitis to subjects with Crohn's disease were performed for all variables, to determine whether there were any significant differences based on diagnosis. Zero-order correlations of dependent with independent variables were conducted. On a more exploratory basis, two multiple regression analyses were conducted. Predictor variables in these analyses were entered hierarchically in relation to their presumed weighting, based on prior empirical investigations.

Ethical Considerations

This study was approved by the Human Research Advisory Committee of the Mount Sinai School of Medicine. All subjects participated voluntarily and gave informed consent to participate. Subjects gave explicit consent for their treating physician to provide information to the senior author about diagnosis, course of illness, and disease severity.

Results

Results of *t*-tests comparing subjects with ulcerative colitis to subjects with Crohn's disease revealed no significant differences based on diagnosis. Thus, these groups were combined in all statistical analyses.

Demographic Characteristics

The mean duration of patient illness was 8 years (range, 2–22 years). The mean age of IBD patients was 24 years (range, 19–32 years) and 88% were unmarried. Subjects' families were characterized by intact parental marriages (13/17) and sibships ranging in size from 0–3 (mean, 1.5 siblings).

Stress and Disease Activity

Strong negative correlations were found between recent disease activity and family stress (Table 2): Physician scores of recent disease severity from the Inflammatory Bowel Disease Severity Index were inversely related to family Total Recent Life Change scores on the FILE (r = –0.79, p<0.01) and to family Total FILE scores (r = –0.78, p<0.01). This indicates that high recent disease activity is associated with low family stress, while low recent disease activity is associated with high family stress. Consistent with these results, multiple regression analysis showed recent family-wide stress to be a significant negative predictor of recent disease severity (p<0.01) (Table 3). Notably, patient stress (as measured by the SRE) was not predictive of recent disease severity (p = 0.32).

Stress and Patient Coping

A high frequency of stressful life events in the life of the patient and in the lives of individual family members, recently and over time, was found to be associated with reduced ability by the patient to cope effectively with IBD (Table 2). Patient coping (measured by the Experience Scale) was significantly associated with the patient's individual SRE scores for the past 6 months (r = –0.60, p<0.05) and the past 2–3 years (r = –0.63, p<0.01). It was also strongly associated with SRE scores averaged across family members for the past 6 months (r = –0.72, p<0.01) and for the period extending back 2–3 years (r = –0.63, p<0.01). Consistent with these results, multiple regression analysis showed patient coping (as measured by the Experience Scale) to be significantly and positively predicted only by SRE measures of recent stress in the life of the IBD patient (p<0.01) and the family as a whole (p<0.01) (Table 3).

Family Communication and Disease Severity

It is noteworthy that recent disease severity was found to be significantly and positively correlated with family IBDI Agreement Scores (r = 0.53, p<0.05). Blaming of the IBD patient by the family was found to be significantly correlated with generalized blaming by the family (i.e., the family blaming someone other than the IBD patient for the patient's illness) (r = 0.48, p<0.05).

TABLE 2

Correlations of Dependent with Independent Variables

		Scores Derived from the Inflammatory Bowel Disease Severity Index	
	Individual Coping in IBD+	Recent Severity Index	Past Severity Index
F-COPES(a)	0.348	–0.280	–0.432
Total FILE(b) score	–0.068	–0.775**	–0.340
Total Recent Life Change^	–0.155	–0.794**	–0.206
FES-A(c)	–0.145	0.101	0.327
IBDI Agreement Score	–0.158	0.531*	0.758**
Mean Family Blame	–0.253	–0.043	0.333
Total FSCI(d) score	0.098	–0.431	0.200
IBD Patient			
SRE(e)-Recent	–0.595*	0.109	0.448
SRE-Longitudinal	–0.632**	0.088	0.466
Family			
SRE-Recent	–0.724**	–0.097	0.351
SRE-Longitudinal	–0.630**	–0.073	0.360

* p<0.05

** p<0.01

^ Measured by the Family Inventory of Life Events and Changes

+ Measured by the Experience Scale

(a) Family Crisis-Oriented Personal Evaluation Scale

(b) Family Inventory of Life Events and Changes

(c) Family Environmental Scale - interpersonal relationships dimension

(d) Family Structure and Communication Index

(e) Schedule of Recent Experiences

There was also a significant correlation between self-blame by the IBD patient and the average level of family blame (r = 0.48, p<0.05). Measures of blaming and coping were not significantly related. Multiple regression analysis showed family functioning (measured by the FSCI) to be a significant predictor of recent disease severity (p<–0.01) (Table 3). When zero-order correlations between scores on the 3 dimensions of the FSCI and ratings of disease severity were computed, only the communication dimension reached significance (r = 0.604, p<0.05), indicating that poor communication within the family is associated with high disease severity.

Discussion

This study demonstrated a number of statistically significant associations between patient and family psychological factors, and the illness experience of the patient with IBD. The intent of the study was to investigate the effects of intimate family relationships — spousal, sibling or parental — on IBD coping and symptomatology. However, the study is limited by the small number of subjects

TABLE 3

Summary of Stepwise Regression Analyses for Recent Disease Severity and Psychological Adjustment to IBD

		Recent Severity		Psychological Adjustment$^+$	
	Psychological Variables	R^2	Significance of change in R (p value)	R^2	Significance of change in R (p value)
Step 1	Total Recent Life Change^	0.31	0.006 **	0.02	0.531
Step 2	IBD Patient SRE$^{(e)}$-Recent	0.34	0.321	0.33	0.006 **
Step 3	Recent Severity Index			0.43	0.096
Step 4	FES-A$^{(c)}$	0.34	0.977	0.44	0.477
Step 5	Total FSCI$^{(d)}$ score	0.54	0.013 **	0.44	0.858
Step 6	F-COPES$^{(a)}$	0.54	0.811	0.47	0.429
Step 7	Mean Family Blame	0.54	0.742	0.49	0.476
Step 8	Family SRE-Recent	0.54	0.757	0.70	0.007 **
Step 9	IBDI Agreement Score	0.59	0.262	0.70	0.760

**p<0.01

^ Measured by the Family Inventory of Life Events and Changes

$^+$ Measured by the Experience Scale Table 3. Summary of regression analyses predicting recent disease severity.

$^{(a), (c), (d), (e)}$ See Table 2.

involved and the variability in family composition as described in the **Subjects** section of **Materials and Methods**. We believe that the use of multiple methods of investigation (self-report, physician rating, direct observation and rating) adds confidence to these results, in that it reduces problems associated with shared method variance. Results described should be viewed as exploratory and generative of future research toward refining the effects of relationship context on disease process.

As regards the impact of individual stress, our results are congruent with the longitudinally based finding of North et al. (5), that patient reporting of stressful life events is not significantly correlated with symptomatic exacerbation of IBD. Based on our observations of family stress, it appears that recent disease severity bears an inverse relationship to family stress level. During a flare-up, the family and patient report less experienced family stress than during a period of disease remission. These findings are consistent with Wood's report (7) of decreased disease activity in association with increased marital dysfunction in families in which the affected member has Crohn's disease. It is possible that during periods of intense family stress, patients actually underreport symptomatology to their physicians. If the patient feels that his or her family members cannot deal with another stressor, the actual experience — and certainly the expression — of symptomatology becomes more threatening.

However, an alternative explanation of this unexpected but interesting finding is that the illness may have an organizing effect on an IBD family, providing a demanding but coherent framework in which other sources of family stress take a back seat to the stress of dealing with the patient's illness. Notably, poor family communication was associated with recent severity. Thus, the urgency of dealing with a symptom flare-up and the mobilization of the family around illness management may suppress the expression of individual concerns, so that there is a kind of muted and incomplete discussion of anything other than the medical crisis.

In a kind of symptomatic family rhythm, intensive illness management becomes unnecessary and abates as the affected individual improves, whereas unresolved individual and family issues surface and are destabilizing. In this pattern of symptom oscillation, reduced disease severity is associated with increased interpersonal stress.

Such explanations are consonant with the "psychosomatic family" model, which has proved useful in understanding asthma and anorexia (14). Psychosomatic families typically exhibit conflict avoidance, overly-attuned interpersonal sensitivity (enmeshment), overprotection, and rigidity. In families in which a child has ulcerative colitis, minimization of conflict and disagreement and qualities of isolation and restrictiveness have been described (15). Families in which a child has Crohn's disease have been shown to have an atypically low rate of divorce, hypothetically linked to the psychosomatic family characteristics of conflict avoidance and overprotection (16).

There was a significant positive correlation between both recent and past disease severity, and level of family agreement regarding the etiology and management of IBD. Ongoing high levels of disease may bring the family together in agreement as a way of maintaining order in the face of an unpredictable illness. Agreement may also reflect conflict avoidance, in which potentially useful variation in attitude and perspective among family members is sacrificed to solidarity.

As regards coping with IBD, our results point to a significant relationship between individually based ratings of stress (made both by the patient and by his or her family members) and the patient's self-perceived ability to cope with IBD (such that higher levels of stress are associated with reduced self-perceived competency in coping). Drossman et al. (17, 18) have commented on the importance of coping as a variable distinct from the physical symptoms in IBD. Why and how stress on both the family and individual levels interferes with patient coping with IBD is a matter for continuing study. It is possible that an increase in stress for family members leads to a significant decline in support to the patient, which in turn impairs coping.

Also contrary to our expectations, blame was not a significant predictor of patient coping. However, a pattern of handling blame emerged, indicating that there is a significant association between self-blame by IBD patients and an overall tendency to blame in the family. Blaming provides individuals with an anchored, although inaccurate explanation for the illness, as well as a sense of control over its essential unpredictability. It thus serves as a coping mechanism, albeit with a clearly negative interpersonal effect.

The clinical implications of this study are important, as the results offer insight into the psychosocial setting of the individual with IBD. They may be useful in designing intervention and treatment regimens, and in facilitating family participation and cooperation with treatment.

It is important for a physician treating an individual with IBD to be aware that stress may affect a patient's psychological ability to cope with his or her illness, rather than be directly responsible for an exacerbation of symptoms. Second, although the data are preliminary, the treating gastroenterologist may find it useful to listen for the effect of family influences on patient functioning during clinical flare-ups as well as during times of remission. Additional data are needed to further evaluate the scope and extent of the effect of family influence. However, the physician may find it helpful to make a referral to a family therapist when coping seems impaired in the context of active family involvement.

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Two-Level Thoracic Disc Herniation

NIELS LEVI, M.D., AND KJELD DONS, M.D.

Abstract

We report a rare case of two-level thoracic disc herniation that occurred in a 48-year-old woman. She was referred with a 10-month history of pain on the right side of the thorax. On examination, she had hypoesthesia and hypalgesia in the right T6–T8 dermatomes. An MRI scan revealed a large herniated disc at the T7/8 level and a smaller herniated disc at the T6/7 level. At surgery, the unilateral transpedicular approach was used, and a large prolapse was removed at the T7/8 level. The T6/7 level was decompressed. The patient made an uneventful recovery. Six months after surgery her pain had disappeared, but she still had hypoesthesia in the right T6–T8 dermatomes. **Key Words:** Thoracic disc herniations, prolapse, transpedicular surgery.

Introduction

THORACIC DISC HERNIATION is uncommon and surgery for thoracic disc herniation represents less than 1–2% of operations for disc disease (1–6). Nevertheless, a great deal of interest has been focused on this disorder because its diagnosis and treatment are difficult (2, 5). Also, thoracic disc herniation is one of the few notable causes of cord compression that are reversible (5). Only 27 cases of two-level thoracic disc herniation have been reported (7–11). We report a rare case of two-level thoracic disc herniation that occurred in a 48-year-old woman.

Case Report

A 48-year-old woman was referred with a 10-month history of pain on the right side of the thorax. She had no previous medical history apart from osteomyelitis in her left arm when she was a child. The only medication she was taking was carbamazepine, which had very little effect on her thoracic neuralgia. On examination, she had hypoesthesia and hypalgesia in the right T6–T8 dermatomes. Motor and sensory nerve function in the arms and legs was normal and she had no sphincter disturbance. An MRI scan with sagittal T1 and T2 weighted image with gadolinium contrast revealed a large herniated disc at the T7/8 level and a smaller herniated disc at the T6/7 (Fig.). At surgery, the unilateral transpedicular approach was used, and a large prolapse was removed at the T7/8 level. The T6/7 level was decompressed. Histologic examination of the disc material showed fibrocartilage tissue. The patient made an uneventful recovery. Six months after surgery her pain had disappeared, but she still had hypoesthesia in the right T6–T8 dermatomes.

Discussion

The diagnosis of thoracic herniation is considered to be difficult and the clinical presentation can be perplexing, since a wide range of manifestations is possible (7). Differential diagnosis from spinal cord tumor, vertebral tumor, spondylitis and cerebrovascular disease is important (1, 3, 7–8). Pain, lower limb numbness and weakness, paresthesia and sphincteral dysfunction are the most common symptoms (8). Multiple thoracic disc herniations have been reported at every level, but they occur most frequently at the lower levels (10). In a recent review of 26 cases, it was found that two contiguous vertebral discs were affected in 80% of cases, with 55% from

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From the Department of Neurosurgery, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark.

Address correspondence to Niels Levi, MD, Vermlandsgade 77, Copenhagen 2300 S, Denmark.

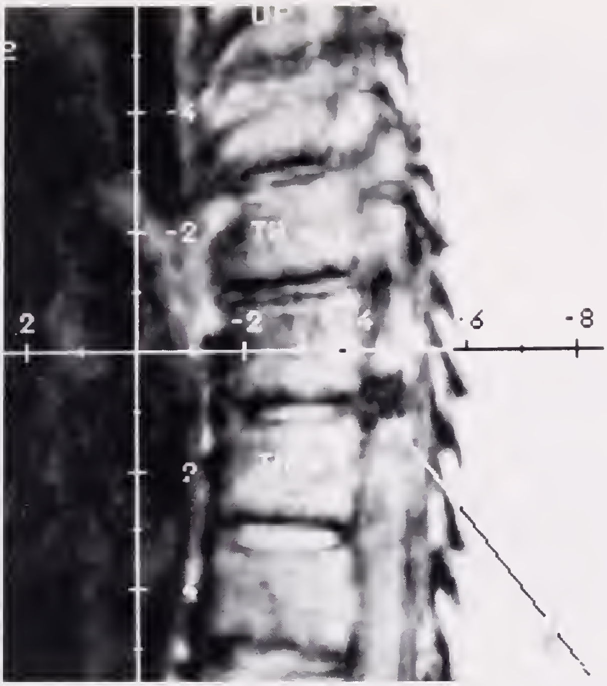

Fig. An MRI scanning with sagittal T1 and T2 weighted image with gadolinium contrast showing a large herniated disc at the T7/8 level and a smaller herniated disc at the T6/7 level diagnosis.

T7–T10 (8). Females seem to be affected more frequently than males, younger patients more often than older ones (8). Magnetic resonance imaging (MRI) has surpassed CT/myelography and is now the diagnostic examination of choice in the detection of thoracic disc disease (6). Most herniation will be detected on the sagittal T1-weighted sequence, but axial T1-weighted or sagittal T2-weighted images may be necessary for detection of all lesions (6). However, difficulty in differentiating thoracic disc herniation and osteophytes can be encountered, and if operative treatment is considered, MRI should be supplemented by myelography combined with CT to select the optimal surgical approach (4). Early treatment with decompressive laminectomy, especially in association with disc removal, has proven disappointing and has been discredited as surgical therapy for thoracic disc herniation, because of an unacceptably high incidence of spinal cord damage (1–5). This seems to be due to the narrowness of the subarachnoid space around the thoracic spinal canal (3). When surgery is attempted in this situation, the slightest manipulation of the cord in order to achieve disc excision can be hazardous (3). To avoid cord manipulation, more anterior and lateral procedures have been developed (2–4). Principally, these are the anterolateral or transthoracic approach, the posterolateral approaches (including the transpedicular, transversoarthropedicular and costotransversectomy) and the lateral extracavitary approach (1–5). All are effective, and postoperative neurological deterioration is unusual (1, 3, 4). The transpedicular approach, originally described by Patterson and Arbit in 1978 (12), has been found to be an effective and safe method of surgical decompression (4). The relative simplicity of the procedure is one of its advantages, since transthoracic approaches may be problematic for patients in poor medical condition (4).

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Judaism, Genetic Screening and Genetic Therapy

Fred Rosner, M.D., F.A.C.P.

Abstract

Genetic screening, gene therapy and other applications of genetic engineering are permissible in Judaism when used for the treatment, cure, or prevention of disease. Such genetic manipulation is not considered to be a violation of God's natural law, but a legitimate implementation of the biblical mandate to heal. If Tay-Sachs disease, diabetes, hemophilia, cystic fibrosis, Huntington's disease or other genetic diseases can be cured or prevented by "gene surgery," then it is certainly permitted in Jewish law.

Genetic premarital screening is encouraged in Judaism for the purpose of discouraging at-risk marriages for a fatal illness such as Tay-Sachs disease. Neonatal screening for treatable conditions such as phenylketonuria is certainly desirable and perhaps required in Jewish law. Preimplantation screening and the implantation of only "healthy" zygotes into the mother's womb to prevent the birth of an affected child are probably sanctioned in Jewish law. Whether or not these assisted reproduction techniques may be used to choose the sex of one's offspring, to prevent the birth of a child with a sex-linked disease such as hemophilia, has not yet been ruled on by modern rabbinic decisions. Prenatal screening with the specific intent of aborting an affected fetus is not allowed according to most rabbinic authorities, although a minority view permits it "for great need." Not to have children if both parents are carriers of genetic diseases such as Tay-Sachs is not a Jewish option. Preimplantation screening is preferable. All screening test results must remain confidential. Judaism does not permit the alteration or manipulation of physical traits and characteristics such as height, eye and hair color, facial features and the like, when such change provides no useful benefit to mankind. On the other hand, it is permissible to clone organisms and microorganisms to facilitate the production of insulin, growth hormone, and other agents intended to benefit mankind and to cure and treat diseases.. **Key Words:** Premarital screening, genetic diseases, Judaism

Introduction

Genetic information about a person's health and health prospects can be inferred from family history or by direct genetic testing. Such testing can involve sophisticated molecular analysis for the mutant gene (e.g., cystic fibrosis) or simple biochemical (e.g., hypercholesterolemia), enzymatic (e.g., Tay-Sachs disease), hematological (e.g., Sickle cell diseases), or chromosomal (e.g., Down's syndrome) analysis of blood or body fluids or tissues.

Points to consider before embarking on carrier screening programs include the nature and frequency of the disorder and availability and effectiveness of treatment, community perception of the disorder and attitudes to screening, motivation for screening, how the test is done and what the results mean, obtaining informed consent and maintaining confidentiality of results, when to screen, education before screening, possible stigmatization and discrimination, and the organization of the screening program (1). The Council on Ethical and Judicial Affairs of the American Medical Association has addressed ethical issues related to prenatal genetic testing (2), genetic testing by employers (3) and insurers (4), and carrier screening for cystic fibrosis and other disorders (5).

How does society control the way genetic information is obtained and used? How does society monitor and review genetic screening programs? What criteria should be set to make maximum use of the potential good that the Human Genome Project offers, without infringing on the ethical and legal principles of privacy, autonomy, beneficence, confidentiality and nondiscrimina-

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From the Director, Department of Medicine, Mount Sinai Services at Queens Hospital Center, Jamaica, NY, and Professor of Medicine, Mount Sinai School of Medicine, New York, NY. Address correspondence to Fred Rosner, M.D., F.A.C.P., Queens Hospital Center, 82-68 164th Street, Jamaica, NY 11432.

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tion? Who owns genetic information? How should genetic information be used? Who decides which people should be screened? Are there or should there be limits to preimplantation, prenatal and neonatal screening? How should employers and insurers receive and use genetic information? Do we have sufficient data to judge the pros and cons of genetic testing and screening? The Orthodox Jewish community relies on the traditional use of biblical and talmudic law and rabbinic responsa to answer such questions. This approach is described in this essay on traditional Jewish views on genetic issues.

The genetic testing and counseling of children and adolescents is associated with special ethical, legal and psychological implications (6). The risks and benefits of testing have to be assessed to determine whether it is in the child's best interests to be tested (7).

Specific Examples

Breast Cancer

About 5 – 10% of all breast cancer cases are hereditary. The breast cancer genes known as *BRCA1* and *BRCA2* are responsible for most inherited breast cancer, especially in women who develop the disease before the age of 40 years. The *BRCA1* mutation known as 185delAG is found in approximately 1% of the Ashkenazi Jewish population (8) and in 20% of Ashkenazi Jewish women who develop breast cancer before 40 (9) or 42 (10) years of age. This gene is also associated with an increased risk of ovarian cancer. These findings and observations are cause for concern (11). Early interventions may be appropriate in high-risk women who test positive (e.g., more frequent breast examination and mammography, prophylactic mastectomy, hormone prophylaxis). There are many other issues of concern, including confidentiality; access; autonomy; and informing the patient about the implications of a positive or negative test, including its technical accuracy and cost

The availability of a test does not require that it be offered universally. The American Society of Clinical Oncology recommends that cancer predisposition testing be offered only when the person has a strong family history of cancer or very early age of onset of disease, when the test can be interpreted adequately, and when the results will influence the medical management of the patient or family member (12). However, many other prestigious organizations emphasize that *BRCA1* testing remains a research activity for the time being (13). The pitfalls of genetic testing (14) and the psychological issues in testing for breast cancer susceptibility (15) should not be minimized. People at risk must fully understand the risks, benefits, and limitations of genetic testing, the risk of psychological harm, and the possibility of insurance discrimination and subsequent loss of health care coverage, before they undergo testing (16).

Tay-Sachs Disease

Debates continue about the screening of large populations of Jewish people for the carrier state of Tay-Sachs disease to prevent the "inappropriate" marriage of two carriers. Also controversial is the performance of amniocentesis for the prenatal detection of the fatal disease, with the subsequent possible abortion of an affected fetus. Selected termination of affected fetuses may not be acceptable in Judaism, although some rabbis might sanction it. Mass screening programs may produce a psychological burden on those young people who screen positive. Should a carrier of the Tay-Sachs gene refuse to marry an individual who has not been tested? Should a couple break up their engagement if they learn that both are carriers? Should a young person inquire about the Tay-Sachs status of a member of the opposite sex prior to meeting that individual on a social level? Must a person who knows he or she is a carrier divulge this fact to an intended spouse?

The stigma of being a carrier of the Tay-Sachs gene may not be fully appreciated. Misinformed or uninformed people may shun and ostracize such carriers. Job and insurance discrimination is also possible if confidentiality of testing results is not assured. If the purpose of Tay-Sachs screening is to provide information and genetic counseling about mating and reproductive options, few will oppose screening. If the purpose, however, is to suggest prenatal diagnosis with the specific intent of recommending abortion of affected fetuses, religious and moral objections might be raised. Preimplantation diagnosis of *in vitro* fertilized eggs, with the discarding of affected zygotes, if any, avoids the issue of pregnancy termination since pregnancy in Judaism does not begin until zygote implantation into the wall of the uterus.

Genetics and Eugenics in Classic Jewish Sources

Ancient Jewish writings, including the Bible and Talmud, are not devoid of material relating to genetics. One writer describes in some detail how the laws of Mendelian genetics were applied by Jacob in the biblical narrative (Genesis 30:32 ff) of the speckled and spotted sheep (17). Hemophilia and its precise genetic transmission is described in the Talmud (Yebamot 64b). The Sages in the Talmud and subsequent rabbinic authorities had a remarkable knowledge of the genetics of this sex-linked disorder (18). All rabbis recognized that females transmit the disease but do not suffer from it. A few rabbis also considered the possibility of its transmission through males.

Elsewhere (Ketubot 10b), the Talmud portrays a family whose women had hereditary absence of menstruation and no blood of virginity, and were obviously childless. The exact nature of the anatomical or physiological abnormality is not described.

It is prohibited in Jewish law to marry a woman from a family of epileptics or lepers (Yebamot 64b; Maimonides' *Mishneh Torah, Issurei Biyah* 21:30; Karo's *Shulchan Aruch, Even Haezer* 2:7) lest the illness be genetically transmitted to future generations. According to Rashi (Yebamot 64b), any hereditary disease is included in this category. This talmudic ruling (19) "may well represent the first eugenic enactment, and the only legislative bar to the procreation of a diseased progeny, in ancient and even medieval times." On the basis of the higher frequency of defective births resulting from unions among blood relatives, Rabbi Judah the Pious, in his ethical will, prohibited marriages between first cousins and between uncles and nieces. Yet such marriages are sanctioned in the Bible and expressly encouraged in the Talmud (Yebamot 62b and Sanhedrin 76b). Since consanguineous marriage increases the probability of birth defects, some rabbis ban such marriages (20, 21), while others strongly caution against them (22–24).

Genetic disease was recognized by Maimonides, who prescribes a regimen of health for all Jews to remain healthy, since one cannot serve the Lord when one is ill (*Mishneh Torah,* Deot 4:1). He guarantees that anyone who follows his regimen will be healthy all his life, except for those who were born with hereditary or genetic defects (*Ibid.* 4:20).

The Genome Project and Judaism

Is the genome project an encroachment on the Divine plan for this world by interfering with nature as God created it? Would genetic engineering tamper with the Divine arrangement of Creation? Although one rabbi answers in the affirmative (25), most rabbis consider the acquisition of knowledge for the sake of finding cures for human illnesses to be divinely sanctioned, if not in fact mandated. God blessed mankind with the phrase: *replenish the earth and subdue it* (Genesis 1:28). This phrase is interpreted by Nachmanides (Ramban) to mean that God gave man dominion over the world to use animals and insects and all creeping things for the benefit of mankind (Ramban, Genesis 1:28). To subdue the earth, according to Samson Raphael Hirsch (Hirsch, Genesis 1:28), is to master, appropriate, and transform the earth and its products for human purposes. To have *dominion over the fish and over the birds and over every living thing on earth* (Genesis 1:28) means to use them for the benefit of mankind. The pursuit of scientific knowledge does not constitute prohibited eating from the tree of knowledge (Genesis 2:17). Whatever is good for mankind must be permissible and praiseworthy. However, good often is not pure good, but may contain potentially dangerous elements. Although the genome project is intended to cure diseases, it has raised many concerns.

In the general introduction to his *Commentary on the Mishnah* (26), Moses Maimonides discusses the existence and purpose of all living and inanimate things in the world. He clearly enunciates the thesis that the purpose of everything that God put on this earth is to serve mankind. Thus, scientific experiments on laboratory animals, during the course of medical research that might find cures for human illnesses, are sanctioned in Jewish law as legitimate utilization of animals for the benefit of mankind (27). However, whenever possible, pain or discomfort should be avoided or minimized in order not to transgress the prohibition in Jewish law against cruelty to animals.

King David said that *The heavens are the Lord's heavens but the earth He has given to mankind* (Psalms 115:16), further supporting the concept that knowledge and its pursuit are legitimate activities for human beings and not considered an encroachment upon Divine prerogatives. Thus, therapeutic genetic engineering and gene therapy that may result from the knowledge derived from the genome project do not undermine God's creation of the world by manipulating nature (Ramban, Leviticus 19:19). On the contrary, it is a legitimate modification of the natural order. The use of scientific knowledge to benefit mankind is biblically mandated (Ramban, Genesis 1:28). The use of such knowledge to heal illness and cure disease is also allowed biblically, based on the talmudic interpretation (Baba

Kamma 85a) of the phrase *and heal he shall heal* (Exodus 21:19), or even biblically mandated, based on Maimonides' interpretation (*Mishnah Commentary*, Nedarim 4:4) of the biblical obligation to restore a lost object (Deuteronomy 22:2) to include the restoration of one's lost health. The healing of illness includes the use of genetically engineered medications such as insulin and various antibiotics. The cure of disease by gene therapy, if possible, is also sanctioned in Jewish law.

Genetic Screening and Judaism

Many years ago, Rabbi Moshe Feinstein (28) was asked whether or not it is advisable for a boy or girl to be screened for Tay-Sachs disease and, if it is proper, at what age the test should be performed. His answer was:

> . . . it is advisable for one preparing to be married, to have himself tested. It is also proper to publicize the fact, via newspapers and other media, that such a test is available. It is clear and certain that absolute secrecy must be maintained to prevent anyone from learning the result of such a test performed on another. The physician must not reveal these to anyone . . . these tests must be performed in private, and, consequently, it is not proper to schedule these tests in large groups as, for example, in Yeshivas, schools, or other similar situations.

Rabbi Feinstein (29) also points out that most young people are quite susceptible to nervous tension or psychological stress and, therefore, young men (below age 20) and women (below age 18) not yet contemplating marriage should not be screened for Tay-Sachs disease. Finally, Rabbi Feinstein strongly condemns abortion for Tay-Sachs disease and even questions the permissibility of the amniocentesis which proves the presence of a Tay-Sachs fetus, since amniocentesis is not without risk, albeit small.

Rabbi J. David Bleich (30) indicates that the elimination of Tay-Sachs disease is, of course, a goal to which all concerned individuals subscribe. He points out, however, that the obligation with regard to procreation is not suspended simply because of the statistical probability that some children of the union may be deformed or abnormal. While the couple may quite properly be counseled with regard to the risks of having a Tay-Sachs child, according to Jewish law (Halachah) the failure to bear natural children is not a viable alternative. He further voices concern that if the fetus is found to have Tay-Sachs disease by prenatal testing, abortion may not be sanctioned in Jewish law. Rabbi Bleich concludes that screening programs for the detection of carriers of Tay-Sachs disease "are certainly to be encouraged." He suggests that the most propitious time for such screening is childhood or early adolescence, since early awareness of a carrier state, particularly as part of a mass screening program, is advantageous. He is critical of Rabbi Waldenberg, pointing out that the latter's permissive ruling on abortion for Tay-Sachs disease is contrary to the decisions of other contemporary rabbinic scholars, including Rabbi Feinstein.

Two methods now exist for totally eliminating the need for prenatal screening for Tay-Sachs disease and thus averting the serious halachic objections to abortion if the fetus should be found to be affected. The first method is to perform confidential premarital screening and to strongly discourage the marriage of two carriers. This approach, widely utilized in many Orthodox Jewish communities, is sponsored by the Dor Yeshorim organization (160 Wilson Street, Brooklyn, NY 11211, [718] 384-6060), which claims to have tested more than 70,000 people and identified more than 100 at-risk couples, who were advised not to marry. In the United States, the program has significantly reduced the number of Jewish babies born with the disease. And in Israel, a similar screening program has resulted in no Tay-Sachs children being born to newlywed couples in the ultra-Orthodox Ashkenazi Jewish community in over 10 years (31).

The second method of preventing the birth of a baby with Tay-Sachs disease, hemophilia, or Huntington's disease is to perform preimplantation screening of *in vitro* fertilized zygotes if both husband and wife are known carriers and to use only the unaffected ones for implantation. Whether one may screen these *in vitro* fertilized zygotes for genetic diseases has yet to be ruled on decisively by modern rabbinic authorities. However, *in vitro* fertilization is sanctioned by many rabbis for couples who cannot conceive in the normal way (32). Moveover, the discarding of the affected zygotes would not be considered abortion, since Jewish law considers life to begin only when the living embryo has become implanted in the mother's womb and continues to grow.

It is not clear whether Judaism sanctions genetic screening for diseases for which no effective treatment yet exists. The rabbis are greatly concerned about the emotional burden (*tiruf hadaat*) that such knowledge may place upon a person found to have the gene for Huntington's disease in the presymptomatic stage. Judaism would not sanction amniocentesis or chorionic villi sampling to rule out Huntington's disease if the only purpose is to abort the fetus if it is found to be affected. However, preimplantation screening of Huntington's disease and choosing only unaffected zygotes for implantation may be permissible, to prevent the birth of an affected child, as described above for the prevention of Tay-Sachs disease. The same permissive view might apply to the prevention of hemophilia births by preimplantation screening.

Newborn screening for treatable diseases such as phenylketonuria and congenital hypothyroidism should certainly be done. Judaism subsumes such testing under the biblical and rabbinic mandates to seek healing from the medical profession.

Judaism requires that confidentiality of test results for all types of genetic screening be maintained. The prohibitions in Judaism against talebearing (Leviticus 19:16) and evil gossip (Psalms 34:14) are discussed at length in the Talmud (Yoma 4b, Sanhedrin 31a) and in the Codes of Jewish law such as Maimonides' *Mishneh Torah* (Deot 7:2). An entire book was written on this subject by Rabbi Israel Meir Hakohen of Radin, popularly known as "Chafetz Chayim" (33). Whenever the physician obtains confidential medical information, genetic or otherwise, he is forbidden to disclose that information or share it with anyone, including the patient's family and even professional colleagues, if no benefit to the patient would result. However, if keeping confidence might impact adversely on the health of another person, the latter may be informed. In Jewish law, a person who is the carrier of a serious and potentially lethal genetic disorder is obligated to divulge that information to a prospective spouse.

More difficult to resolve is the question as to whether or not an Ashkenazi Jewish woman with the gene for breast cancer *BRCA1* or *BRCA2* is obligated to tell that to a prospective spouse or to her husband if she is already married. Modern rabbinic authorities have not yet ruled on whether it is even appropriate to test for that gene in all Jewish women. It may be reasonable to do so in women with very strong family histories of breast cancer. But to what end? If they are found not to have the gene, the risk of developing breast cancer is still high. But women found to be positive for the gene may wish to take action such as more frequent mammography, prophylactic hormonal treatment, or even prophylactic mastectomies. Current rabbinic authorities need to address how such matters might impact on therapeutic choices within the context of Judaism.

Gene Therapy and Genetic Engineering

The literature in Jewish law on gene therapy and genetic engineering is very sparse indeed. Two rabbinic articles with genetic engineering in their titles (34, 35) deal primarily with artificial insemination, *in vitro* fertilization and surrogate motherhood, and only briefly mention cloning. The production of hormones such as insulin and erythropoietin, and antibiotics and other therapeutic substances, by genetic engineering through recombinant DNA technology is certainly permissible in Jewish law, because nature is being used properly by man to his benefit in the treatment and cure of illnesses. Gene therapy, such as the replacement of the missing enzyme in Tay-Sachs disease and the missing hormone in diabetes, and the repair of the defective gene in hemophilia or Huntington's disease, if and when these become scientifically feasible, are also probably sanctioned in Jewish law, because they are done with the intention of restoring health, and preserving and prolonging life. The technical medical problems of modifying the defective gene or genes in an individual sperm, ovum or zygote by gene surgery and implanting the replaced or repaired genes into the mother in order to produce a healthy child have not yet been surmounted. However, assuming such surgery can be performed successfully, gene surgery will probably be sanctioned by rabbinic authorities as a legitimate implementation of the mandate on physicians to heal the sick.

Another argument favoring the permissibility of gene surgery or genetic manipulation is the fact that neither the sperm nor ovum nor even the fertilized zygote is a person. Thus, gene manipulation is not considered to be tampering with an existing or even a potential human being, since that status in Jewish law is only bestowed upon a fetus implanted in the mother's womb. One can also argue that any surgery performed on a live human being must certainly be permitted on a sperm or ovum or fertilized zygote. For example, if a surgical cure for hemophilia, Tay-Sachs disease or Huntington's disease were possible, it would surely be permissible. Hence, it should certainly be permissible to cure or prevent these diseases by gene surgery.

Rabbi Moshe Hershler (25) warns against blinding ourselves to the potential of genetic engineering and gene therapy, which is no longer a dream or a fantasy but becoming a medical and scientific reality. Hershler raises the question of the permissibility (or lack thereof) of experimenting with gene therapy to try to save the life of a child with thalassemia or Tay-Sachs disease if the unsuccessful outcome of the experimentation would be a shortening of the child's life. Hershler is of the opinion that gene therapy and genetic engineering may be prohibited because "he who changes the [Divine] arrangement of creation is lacking faith [in the Creator]," and he cites as support for his view the prohibition against mating diverse kinds of animals, sowing together diverse kinds of seeds, and wearing garments made of wool and linen (Leviticus 19:19). This line of reasoning is rejected by Rabbis Shlomo Zalman Auerbach and Yehoshua J. Neuwirth (36), since genetic engineering does not seem to be comparable to the grafting of diverse types of animals or seed. The main purposes of gene therapy are to cure disease, restore health, and prolong life, all goals within the physician's Divine license to heal. Gene grafting is no different than an "organ graft," such as a kidney or corneal transplant, which nearly all rabbis consider permissible.

Ethical and halachic problems associated with genetic engineering include "speciation." Does a certain species lose its identity if other genes are introduced into it? Would the citron or *ethrog* (*Citrus medica* Linn) used on the Tabernacles holiday (Sukkot) for religious purposes lose its identity if lemon genes were introduced into it? How many transplanted lemon genes are needed to consider the *ethrog* to be a lemon? Can the rabbinic concept of nullification (*bitul*), whereby one part of a prohibited substance becomes nullified if mixed within sixty parts of a permitted substance, be applied to this situation? Another example is the need for fins and scales for fish to be kosher for consumption. If genes introduced in a scaleless catfish induce scalation, does the catfish then become a kosher fish? Yet another example is the conversion by genetic engineering of annual plants into perennials. The latter are not subject to some of the laws of the Sabbatical year. Thus, perennial wheat, corn or tomatoes would be permitted in Jewish law even if grown during the Sabbatical year. These problems and issues have not yet been decisively discussed and resolved by current halachic authorities.

It seems clear that genetic engineering and gene therapy can and should be used to promote the human condition and treat, cure and prevent disease. But should these techniques be allowed to alter human traits such as eye color, height, personality, intelligence and facial features? The Talmud relates (Pesachim 54a) that God inspired Adam with a type of Divine knowledge, and he took two heterogeneous animals and crossed them and created a mule. Elsewhere, the Talmud asks (Chullin 7b) why they are called mules (Hebrew *yemim*) and answers "because they cast fear [Hebrew *emah*] upon men." This inappropriate use of nature by Adam is what Ramban condemns in his biblical commentary (Leviticus 19:19) as "changing and denying the Divine creation of the world."

There is no specific halachic prohibition against attempting to clone a human being. An example of the creation of an artificial human being or *golem* is cited in the Talmud (Sanhedrin 65b). The *golem*, however, was not formed in and born from a woman's womb. It was therefore not considered to be human and was destroyed without that being considered an act of murder. A cloned human being, on the other hand, has the full status of a human being. Although it is not prohibited in Jewish law to clone a human being, one should be very cautious and not do so indiscriminately. Many potential scientific and Jewish legal problems would first need to be dealt with. The risks of producing serious birth defects through human cloning are not known. In Judaism, paternity is determined by the sperm (37). But in human cloning, no sperm is used; so who is the father?

The possible deleterious effects of genetic engineering and gene therapy are not yet fully known. And in addition to the medical and scientific aspects of genetic engineering, DNA recombinant research and human cloning, the spiritual and theological aspects also require exploration. Rabbis must examine these issues from the Jewish viewpoint and offer halachic guidance to the medical and lay communities.

Conclusion

Genetic screening, gene therapy and other applications of genetic engineering are permissible in Judaism when used for the treatment, cure, and prevention of disease. Such genetic manipulation is not considered to be a violation of God's natural law but a legitimate implementation of the biblical mandate to heal. According to Jewish law, if Tay-Sachs disease, diabetes, hemophilia, cystic fibrosis, Huntington's disease or other genetic diseases can be cured or prevented by "gene surgery," it is certainly permitted.

Genetic premarital screening is encouraged in Judaism for the purpose of discouraging at- risk marriages for a fatal illness such as Tay-Sachs disease. Neonatal screening for treatable conditions such as phenylketonuria is certainly desirable and even required under Jewish law.

Preimplantation screening and the use of only unaffected zygotes for implantation into the mother's womb to prevent the birth of an affected child is probably sanctioned in Jewish law. Whether or not these assisted reproduction techniques can be used to choose the sex of one's offspring to prevent the birth of a child with a sex-linked disease such as hemophilia has not yet been ruled on by modern rabbinic authorities (38). Prenatal screening with the specific intent of aborting an affected fetus is not allowed according to most rabbinic authorities, although a minority view permits it "for great need." Not to have children if both parents are carriers of genetic diseases, such as Tay-Sachs, is not a Jewish option. Preimplantation screening is preferable. All screening test results must remain confidential. To improve physical traits and characteristics such as height, eye and hair color, facial features and the like, is frowned upon in Judaism if it serves no useful medical or psychological purpose. The cloning of man is not prohibited as a violation of the Divine arrangement of the world and the creation of man in the image of God. However, Lord Rabbi Immanuel Jakobovits (19) expresses sentiments which we should all take to heart:

> It is indefensible to initiate uncontrolled experiments with incalculable effects on the balance of nature and the preservation of man's incomparable spirituality without the most careful evaluation of the likely consequences beforehand . . . . "Sparepart" surgery and "genetic engineering" may open a wonderful chapter in the history of healing. But without prior agreement on restraints and the strictest limitations, such mechanization of human life may also herald irretrievable disaster resulting from man's encroachment upon nature's preserves, from assessing human beings by their potential value as tool-parts, sperm donors or living incubators, and from replacing the matchless destiny of the human personality by test-tubes, syringes and the soulless artificiality of computerized numbers. Man, as the delicately balanced fusion of body, mind and soul, can never be the mere product of laboratory conditions and scientific ingenuity.

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The Mount Sinai Hospital Clinicopathological Conference

MICHAEL E. BLAM, M.D., Editor

A 50–Year–Old Male with Diabetes Mellitus, Peripheral Neuropathy, Hypothyroidism, Obesity, Sleep Disorder, Pickwickian Syndrome, and Heart Failure

NOEL MANN, M.D., MICHAEL KIM, M.D., AND DUSHYANT P. PUROHIT, M.D.

Presentation of a Case

Dr. Noelle Mann[1]: NR is a 50-year-old male with a medical history significant for diabetes mellitus, peripheral sensory neuropathy, difficulty in sleeping, chronic daytime somnolence, exertional dyspnea, two pillow orthopnea, early satiety, and hypothyroidism. He denied tobacco use, excessive alcohol consumption, and recent travel. He was admitted to The Mount Sinai Hospital in early January 1997 for symptoms of increasing shortness of breath, lower extremity edema, and increasing abdominal girth which had failed to respond to increasing doses of oral furosemide. Four years previously, he had been admitted to another hospital, with similar symptoms, and was diagnosed with heart failure. He was treated with diuretics at that time and had lost more than 100 pounds. Despite therapy with diuretics, he noticed weight gain, and recurrence of increasing shortness of breath, lower extremity edema, and abdominal girth since April 1996.

On admission, his medications included: glipizide 20 mg per day by mouth, furosemide 400 mg per day by mouth, digoxin 0.25 mg per day by mouth, aspirin one-half tablet (162.5 mg) per day by mouth, and oral potassium supplements 40 mEq in the morning and evening, and 20 mEq at bedtime. Levothyroxine replacement therapy had been stopped in November 1996.

On admission, the patient was found to be an obese male in no acute distress. He was afebrile with a blood pressure of 130/90 mm Hg. The pulse and respiratory rates were 80 and 18 per minute respectively. Physical examination was notable for jugular venous distention at 8 – 10 centimeters above the angle of Louis (read with the patient sitting at a 45-degree angle to horizontal). Auscultation of the lungs revealed no abnormal breath sounds. Examination of the heart revealed audible first and second heart sounds with a loud P_2. The abdomen was distended and tense with shifting dullness. Examination of the extremities showed pitting edema to both mid-thighs.

Initial laboratory values are found in Table 1. The chest roentgenogram (Fig. 1) on admission revealed a normal cardiac silhouette and pulmonary vasculature, a tortuous aorta, sharp costophrenic angles, no infiltrates, and a slight prominence of interstitial lung markings at both lung bases. The electrocardiogram (Fig. 2) showed

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From the Department of Medicine, Mount Sinai School of Medicine, New York, NY.

Address correspondence to Michael E. Blam, M.D., Editor, Box 1094, The Mount Sinai Journal of Medicine, One East 100th Street, New York, NY 10029.

Key Words: Pericardium, heart failure, case presentation, review.

[1] Resident Physician, Department of Medicine, The Mount Sinai Hospital, New York, NY.

TABLE 1
Initial Laboratory Values

White blood count	$6.7\text{x}10^3$ cells/mm^3
Hemoglobin	12.8 g/dL
Hematocrit	38%
Platelet count	1.74×10^5 cells/mm^3
Mean corpuscular volume	84.3 μm^3
Red cell distribution width	14.9%
Prothrombin time	14.8 seconds
Partial thromboplastin time	30.6 seconds
Erythrocyte sedimentation rate	25 mm/hr

SERUM CHEMISTRIES

Sodium	136 mmol/L
Potassium	4.4 mmol/L
Chloride	100 mmol/L
CO^2 content	31 mmol/L
Urea nitrogen	39 mg/dL
Creatinine	1.5 mg/dL
Glucose	347 mg/dL
Protein, total	7.4 g/dL
Albumin	4.2 g/dL
Calcium, total	9.4 mg/dL
Magnesium	2.1 mEq/L
Bilirubin, total	0.5 mg/dL
Arterial blood sample:	
pH	7.44
P_{O2} (on room air)	99 mm Hg
P_{CO2}	44 mm Hg

SERUM ENZYMATIC ACTIVITIES

Phosphatase, alkaline	117 IU/L
Alanine aminotransferase (ALT, SGPT)	17 IU/L

SERUM HORMONE VALUES AND THERAPEUTIC AGENTS

Digoxin level	1.1 μg/L
Thyroid Stimulating Hormone	4.7 μU/mL

regular sinus rhythm at a rate of 81 per minute with nonspecific ST-T wave changes and a normal axis.

A PPD test was found to be negative. The patient underwent a trans-esophageal echocardiogram on the day after admission. This was a technically difficult study, but showed normal left ventricular size and function, paradoxical septal motion, and mild pulmonary hypertension. Also noted were mild right ventricular dilatation, moderately decreased right ventricular function, an estimated right ventricular systolic pressure of 36 mm Hg, a patent foramen ovale, minimal mitral and pulmonic regurgitation, and aortic sclerosis. No aortic or intra-cardiac thrombi were found. Three days later, the patient underwent ventilation and perfusion scans of the lungs, which were reported to be normal. A duplex Doppler examination of the lower extremities was negative for venous occlusion. The patient next underwent a nocturnal polysomnography study, which evidenced episodic hypoventilation and apnea (with hypoxemia by pulse oximetry), mild periodic limb movements, and frequent arousals with severely fragmented sleep architecture; these are all consistent with moderate obstructive sleep apnea syndrome. Studies of pulmonary function earlier that day demonstrated probable borderline restrictive impairment with severely reduced maximal inspiratory and expiratory pressures, and a normal diffusing capacity for carbon monoxide. A computed tomogram of the chest showed no evidence of a thickened pericardium (Fig. 3). Right heart catheterization on the 7th hospital day revealed increased pressures in the right atrium, right ventricle, and pulmonary artery (Table 2). A modest decrement in pressure occurred after the administration of aerosolized nitric oxide (Table 2). Abdominal ultrasound on the 14th day revealed ascites with increased echogenicity of the liver and an enlarged spleen.

Concurrently, the patient was treated with intravenous diuretics as well as sodium and fluid restriction. This resulted in a 60-pound weight loss and symptomatic improvement.

On the 15th day, the patient underwent a diagnostic investigation. Three days later, a therapeutic procedure was performed.

Differential Diagnosis

Dr. Michael Kim[2]: In summary, NR is a 50-year-old male with a history of diabetes mellitus with resultant neuropathy, hypothyroidism, and right-sided heart failure. His peripheral edema was resistant to outpatient diuresis.

First, I will discuss the potential source of this patient's edema. Specifically, edema derives from either a cardiac or non-cardiac source. Could this patient have primary kidney or liver disease? An albumin of 4.2 g/dL goes against both nephrotic

TABLE 2
Cardiac Catheterization Data

	RA mm Hg	RV mm Hg	PA mm Hg	PCWP mm Hg
Without NO	19	45/12 (33)	45/20 (28)	16
With NO			32/16 (22)	16

Right heart percutaneous catheterization performed via the internal jugular vein. Values in parentheses are the mean. NO = Nitric oxide; PA = Pulmonary artery; PCWP = Pulmonary capillary wedge pressure; RA = Right atrium; RV = Right ventricle

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[2] Resident Physician, Department of Medicine, The Mount Sinai Hospital, New York, NY.

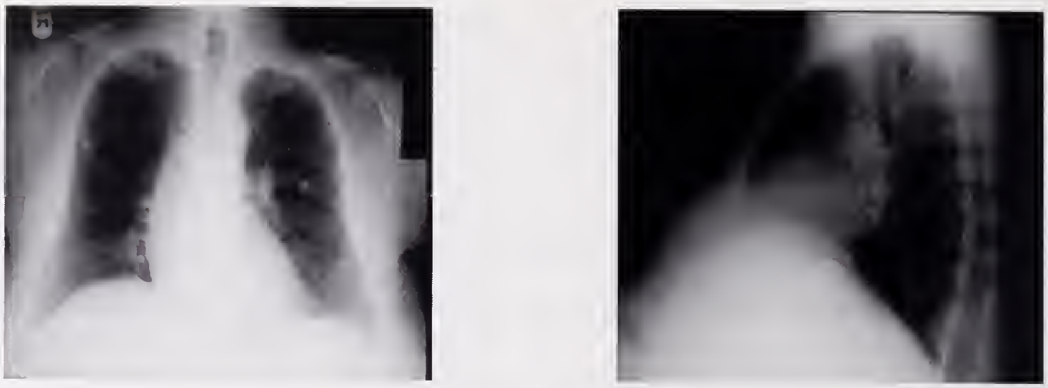

Fig. 1. PA and lateral chest roentgenograms. Cardiac silhouette and pulmonary vasculature are normal. Interstitial lung markings at both bases are slightly increased.

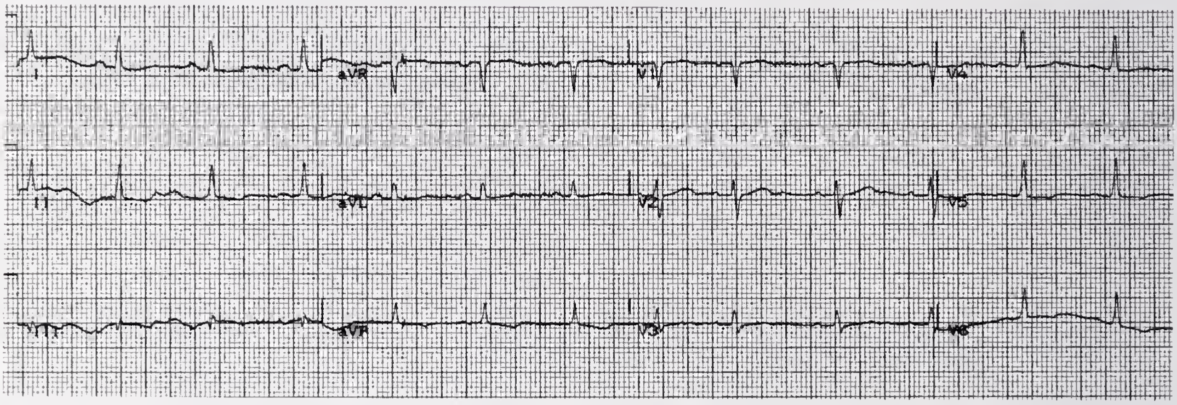

Fig. 2. Electrocardiogram showing normal axis and sinus rhythm at a rate of 81 per minute. ST-T wave changes are non-specific.

syndrome and primary liver disease as the causes of edema. Other findings which argue against liver disease in this patient include normal values for the serum total bilirubin and alanine aminotransferase. The increased prothrombin time, though modestly elevated, does not point strongly towards primary liver disease and could be due to passive congestion of the liver. Finally, jugular venous distention on physical examination is not a specific finding for either the nephrotic syndrome or primary liver insufficiency and is often caused by cardiac pathophysiology.

Table 3 lists the many possible causes of cor pulmonale. Chronic obstructive pulmonary disease and pulmonary thromboembolism are common causes of chronic and acute cor pulmonale. This patient denied the use of tobacco and had no chronic pulmonary complaints. The findings on ventilation and perfusion scanning of the lungs were normal. One consideration was that he might have $\alpha 1$ antitrypsin deficiency, which leads to both liver disease and emphysema. However, the pulmonary function tests did not demonstrate an obstructive pattern, and the chest roentgenogram (Fig. 1) was not consistent with emphysema.

Severe hypothyroidism can cause neuromuscular weakness sufficient to present as cor pulmonale. However, no other symptoms associated with hypothyroidism were mentioned and his serum thyrotropin hormone concentration was normal upon presentation.

Obesity, male gender, daytime somnolence, and difficulty in sleeping are suggestive of obstructive sleep apnea syndrome. Shortness of breath and cor pulmonale are recognized consequences of this syndrome. Polysomnography was consistent with moderate obstructive sleep apnea syndrome, but certain inconsistencies point away from this diagnosis as the principal problem. In particular, ventilation and perfusion scanning of the lungs typically demonstrate mismatch. Arterial blood gases during the daytime are usually consistent with hypercapnia, and secondary erythrocytosis might be expected.

In an effort to explain the patient's presenting signs and symptoms, I will now focus on possible cardiac etiologies. The hallmark of right ventricular failure is systemic venous congestion, as evidenced in this patient by jugular venous distention. Dependent edema, which is very common, is

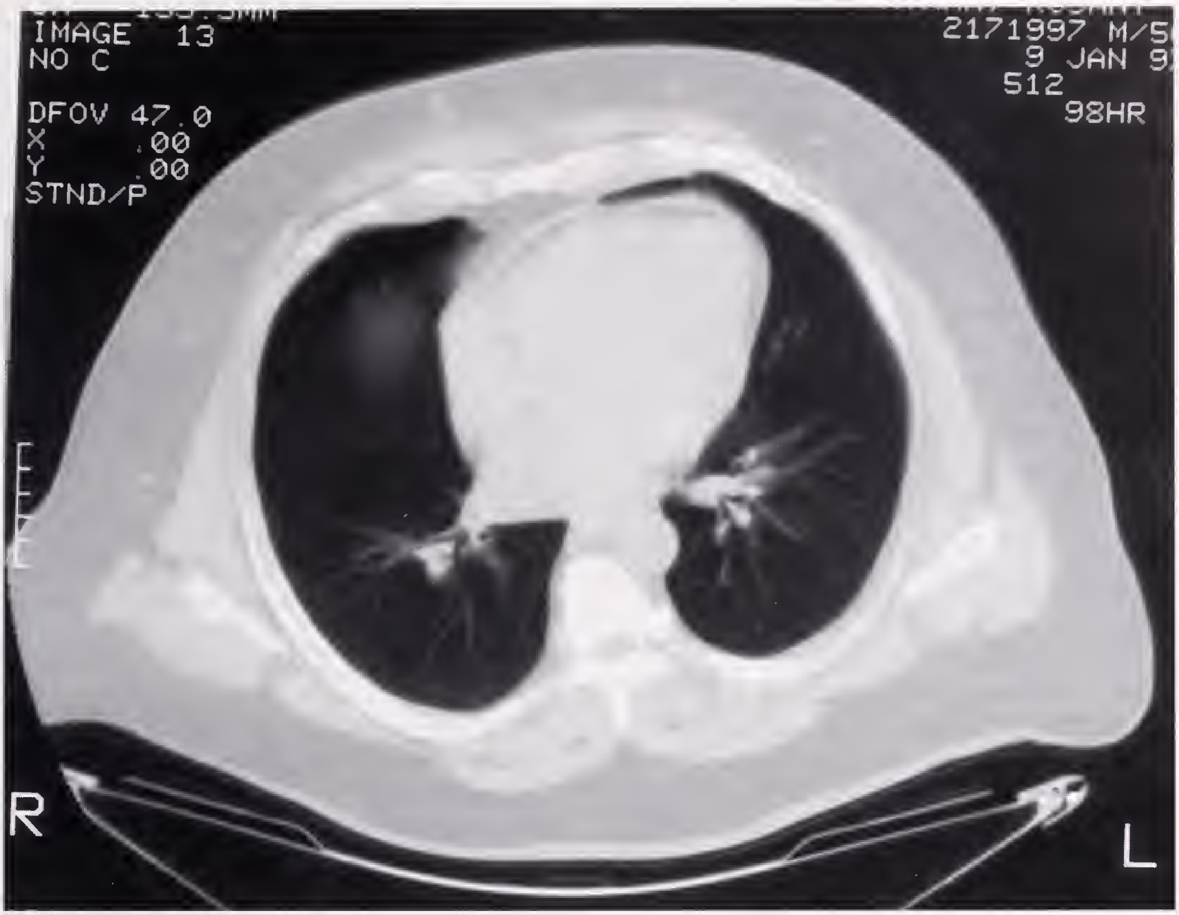

Fig. 3. One slice of a computed tomogram of the chest showing a normal pericardium without any evidence of thickening.

one of the early signs of right ventricular failure. Organ dysfunction from right ventricular failure usually involves the liver first (1). Right upper quadrant abdominal pain, abnormal liver function tests, prolongation of the prothrombin time, and even coma can occur (1). Splenomegaly is possible secondary to right ventricular failure, but it is much less frequent than hepatomegaly and always accompanied by hepatomegaly. Ascites is a late manifestation of heart failure, appearing more frequently in tricuspid regurgitation, restrictive cardiomyopathy, or constrictive pericarditis (1).

Notably, an important clue in this case rests with the admitting chest roentgenogram (Fig. 1). The examination typically reveals impressive cardiomegaly when heart failure is caused by valvular disease and shunts. In contrast, this picture usually is not seen in pericardial and myocardial disease. In this case, the chest roentgenogram reliably excludes a large pericardial effusion. The echocardiogram in this case eliminates the possibility of valvular disease such as tricuspid or pulmonic regurgitation, and supports the logic of focusing on primary myocardial and pericardial disease. The echocardiogram also shows no evidence of either dilated or hypertrophic cardiomyopathy, thereby narrowing the differential in this case to restrictive cardiomyopathy and constrictive pericarditis. As a general rule, one always considers the diagnoses of restrictive cardiomyopathy and constrictive pericarditis when evaluating a patient in heart failure who has a normal heart size on chest roentgenogram.

It should be noted that an important clue regarding restrictive cardiomyopathy or constrictive pericarditis is missing. An abnormal pulsus parodoxus is often appreciated on the physical examination (Table 5).

Cardiomyopathies are classified into three major functional categories (Table 4). Restrictive cardiomyopathy is the least common of these three categories in the Western hemisphere; idiopathic disease and amyloidosis are the most common etiologies worldwide (2). Endomyocardial fibrosis is most prominent in tropical and subtropical Africa, causing 10–20% of deaths due to cardiac disease (2).

The physiological hallmark of restrictive disease is diastolic dysfunction with rigid ventricular

TABLE 3
Etiology of Pulmonary Heart Disease

- I. DISEASES AFFECTING THE PULMONARY VASCULATURE
  - A. Primary Disease of the arterial wall
    - (1) Primary Pulmonary Hypertension
    - (2) Granulomatous pulmonary arteritis
    - (3) Toxin-induced pulmonary hypertension
      - a. Aminorex fumarate
      - b. Intravenous drug abuse
    - (4) Chronic liver disease
    - (5) Peripheral pulmonic stenosis
  - B. Thrombotic disorders
    - (1) Sickle cell diseases
    - (2) Pulmonary microthrombi
  - C. Embolic disorders
    - (1) Thromboembolism
    - (2) Tumor embolism
    - (3) Other embolism (amniotic fluid, air)
    - (4) Schistosomiasis and other parasite diseases
- II. PRESSURES ON PULMONARY ARTERIES BY MEDIASTINAL TUMORS, ANEURYSMS, GRANULOMATA, OR FIBROSIS
- III. DISEASES OF THE NEUROMUSCULAR APPARATUS AND CHEST WALL
  - A. Neuromuscular weakness
  - B. Kyphoscoliosis
  - C. Thoracoplasty
  - D. Pleural Fibrosis
  - E. Sleep apnea syndrome
  - F. Idiopathic hypoventilation
- IV. DISEASES AFFECTING AIR PASSAGES OF THE LUNG AND ALVEOLI
  - A. Chronic obstructive pulmonary diseases
  - B. Cystic Fibrosis
  - C. Congenital developmental defects
  - D. Infiltrative or granulomatous diseases
    - (1) Idiopathic pulmonary fibrosis
    - (2) Sarcoidosis
    - (3) Pneumoconiosis
    - (4) Scleroderma
    - (5) Mixed connective tissue disease
    - (6) Systemic lupus erythematosus
    - (7) Rheumatoid arthritis
    - (8) Polymyositis
    - (9) Eosinophilic granuloma
    - (10) Malignant infiltration
    - (11) Radiation
  - E. Upper airways obstruction
  - F. Pulmonary resection
  - G. High-altitude disease

Adapted with permission. In: Rubin LJ. Introduction. Pulmonary heart disease. Boston: Martinus Nijhoff; 1984. p. 4.

TABLE 4
Classification of the Restrictive Cardiomyopathies

- MYOCARDIAL
  - Noninfiltrative
    - Idiopathic
    - Scleroderma
  - Infiltrative
    - Amyloid
    - Sarcoid
    - Gaucher's disease
    - Hurler disease
  - Storage disease
    - Hemochromatosis
    - Fabry's disease
    - Glycogen storage disease
- ENDOMYOCARDIAL
  - Endomyocardial fibrosis
  - Hypereosinophilic syndrome
  - Carcinoid
  - Metastatic malignancies
  - Radiation

Reprinted with permission from Wynne J and Braunwald E. The cardiomyopathies and myocardities: Heart disease A textbook of cardiovascular medicine. 5th ed. Vol. 2, Philadelphia: W.B. Saunders Co.; 1997. p. 1427. Table 41 – 13.

TABLE 5
Constrictive Pericarditis versus Restrictive Cardiomyopathy

	Constrictive Pericarditis	Restrictive Cardiomyopathy
S_3 gallop	Absent	May be present
Pericardial knock	May be present	Absent
Palpable systolic apical impulse	Absent	May be present
Pericardial calcification	Present 50%	Absent
Pulsus parodoxus	May be present	May be present
Equal RV and LV diastolic pressures	Usually present	LV>RV
Rate of LV filling	80% in first half of diastole	40% in first half of diastole
CAT scan, echo, MRI	Thickened pericardium	Normal pericardium

Modified with permission from Fowler NO: Constrictive Pericarditis. In Fowler NO, editor. The pericardium in health and disease. Mt. Kisco (NY): Futura Publishing Co.; 1985. p. 319.

walls that impede filling. Contractile (systolic) function is usually normal. Hemodynamically, the "square root sign" (3) is a classical feature. This represents the dip and plateau caused by the rapid and deep early decline in ventricular pressure at the onset of diastole, with a rapid rise to a plateau in early diastole. Both pulmonary and systemic venous pressures are elevated, though a larger gradient does point towards a restrictive pattern. Left-sided pressures can be more than 5 mm Hg greater than that of the right side, and the pulmonary artery systolic pressure is usually greater than 50 mm Hg (1).

Symptoms and signs are a direct consequence of the altered physiology. Exercise intolerance is common because tachycardia cannot increase ventricular filling in the face of abnormally stiff ventricles. Chest pain, however, is usually absent. Central venous pressure is elevated, resulting in peripheral edema, liver congestion, ascites, and anasarca. Physical examination reveals an elevated jugular venous pressure and an

S3, S4, or both. Kussmaul sign may be seen (3). The apical impulse remains palpable. The electrocardiogram may show low voltage, atrial fibrillation, and conduction abnormalities (2). The image pattern on echocardiogram may show a "ground glass" appearance suggestive of infiltrative disease, or it may show thickening of the left ventricular wall and an increase in left ventricular mass. Doppler studies may show an expiratory augmentation of hepatic vein diastolic flow reversal (4). The prognosis is indeterminate, though potentially carrying a high mortality rate. No specific therapy is available, but it has been speculated that calcium antagonists may be of some value (1).

Constrictive pericarditis also restricts diastolic filling. The mechanism is limitation of relaxation by a fibrotic, thickened, and adherent pericardium. It usually begins with an initial episode of acute pericarditis, often complicated by a pericardial effusion (5). This effusion undergoes fibrous scarring and thickening, obliterating the pericardial space (5). The most common etiology was formerly tuberculosis, but most cases are now diagnosed as idiopathic (42 percent) (6). Constrictive pericarditis after cardiac surgery and after radiation therapy are well described and have been increasing in frequency over the last decade (6). Rheumatoid arthritis and uremia are also known causes of constrictive pericarditis (6).

Clinical features result from increased systemic venous congestion as in restrictive cardiomyopathy. Common findings are edema, ascites, and passive hepatic congestion along with exertional dyspnea, cough, and orthopnea. Anginal chest pain can occur and is thought to result from underperfusion of coronary arteries that are compressed by the thickened pericardium (5). Kussmaul sign may be appreciated and elevation of the jugular venous pressure is typically observed (3). The arterial pulse may be diminished, but pulsus paradoxus is uncommon (3). A diastolic pericardial knock along the left sternal border may be auscultated (3). Hepatomegaly is usually palpated and anasarca may be found. The chest roentgenogram may show a small, normal, or enlarged heart size. A calcified pericardium may be noted on a lateral film but does not necessarily imply constrictive physiology (7). Electrocardiogram findings include low QRS voltage and P mitrale. Atrial fibrillation is found in fewer than 50 percent of patients (2). Echocardiogram may show pericardial thickening and abrupt displacement of the interventricular septum during early diastole (septal bounce). Doppler studies may also show expiratory augmentation of hepatic-vein diastolic flow reversal (4). Notably, computed tomography and magnetic resonance imaging may show a thickened pericardium (8). Cardiac catheterization of both left and right ventricles will show near equalization of diastolic filling pressures in all chambers and, usually, a right ventricular systolic pressure of greater than 50 mm Hg. A classical "square root" sign, as with the restrictive pattern, may also be present (3).

Constrictive pericarditis occasionally reverses spontaneously (7). Relief of symptoms may require surgical stripping and removal of both layers of the adherent and constricting pericardium. In such cases, elevated venous pressure readings should normalize within the first three months following surgery (9).

The differentiation between restrictive cardiomyopathy and constrictive pericarditis can be very difficult because they both present with overlapping features (Table 5). The exact diagnosis is often found only in the operating room (2). In this particular case, no physical, hemodynamic, or Doppler findings were presented which would aid in the diagnosis. Potential diagnostic investigations include left heart catheterization, endomyocardial biopsy, or pericardial imaging — all to differentiate primary myocardial disease from pericardial disease.

I believe the ultimate diagnosis will be constrictive pericarditis for which a therapeutic procedure was performed. The likely procedure was a thoracotomy with pericardiectomy.

Clinical Diagnosis:

Right-sided heart failure, ? Dilated cardiomyopathy, and ? Obstructive sleep apnea syndrome.

Dr. Michael Kim's Diagnosis:

Constrictive pericarditis.

Pathological Discussion

Dr. Dushyant P. Purohit[3]: An endomyocardial biopsy of the right ventricle was performed, followed by a pericardiectomy. Specimens of myocardium, endocardium, and pericardium were submitted for examination.

Histological examination of the endomyocardial biopsy revealed normal myocardium with no evidence of inflammation, granuloma or necrosis of myocytes, fibrosis of the interstitial tissue, neoplastic infiltration, intracellular storage of abnormal metabolic products, or amyloid deposits. The endocardium was histologically unremarkable.

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[3] Attending Pathologist, Department of Pathology, The Mount Sinai Hospital, New York, NY.

The surgical specimen of the pericardium consisted of three pieces, together measuring 21 x 13 cm and up to 0.6 cm in thickness (normal thickness of pericardium is 0.1 cm). There were no other grossly visible abnormalities, such as hemorrhage, fibrinous deposits, or any focal lesions. Histological examination of the thickened pericardium (Fig. 4) showed marked fibrosis and fusion of the two layers (parietal and epicardial) of the pericardium. Focally the fused surfaces of the pericardial layers showed a thick band of granulation tissue with fibrovascular proliferation, loose cellular stroma and very little mature collagen, suggesting an ongoing repair process. There was no acute or chronic inflammatory infiltrate, fibrinoid necrosis, granuloma, calcification or neoplastic infiltration.

The histopathological findings suggest fibrous pericarditis, consistent with the clinical diagnosis of constrictive pericarditis.

Fibrous pericarditis may develop as a result of varied pericardial abnormalities, including tuberculosis or similar types of granulomatous inflammation, septic pericarditis, trauma, cardiac surgery, irradiation, collagen/autoimmune disease and viral infection (5, 10, 11). More often, however, the etiology is unknown (12). The pericardium did not show histological features of any definitive etiology. While there was no clinical history of pericardial disease, a previous and resolved episode of pericarditis may have occurred. In the past, a common cause of constrictive pericarditis had been tuberculosis. More recently, viral pericarditis has been the major cause. It has been suggested that in some cases of constrictive pericarditis when there is no morphologic evidence of viral infection or any other spe-

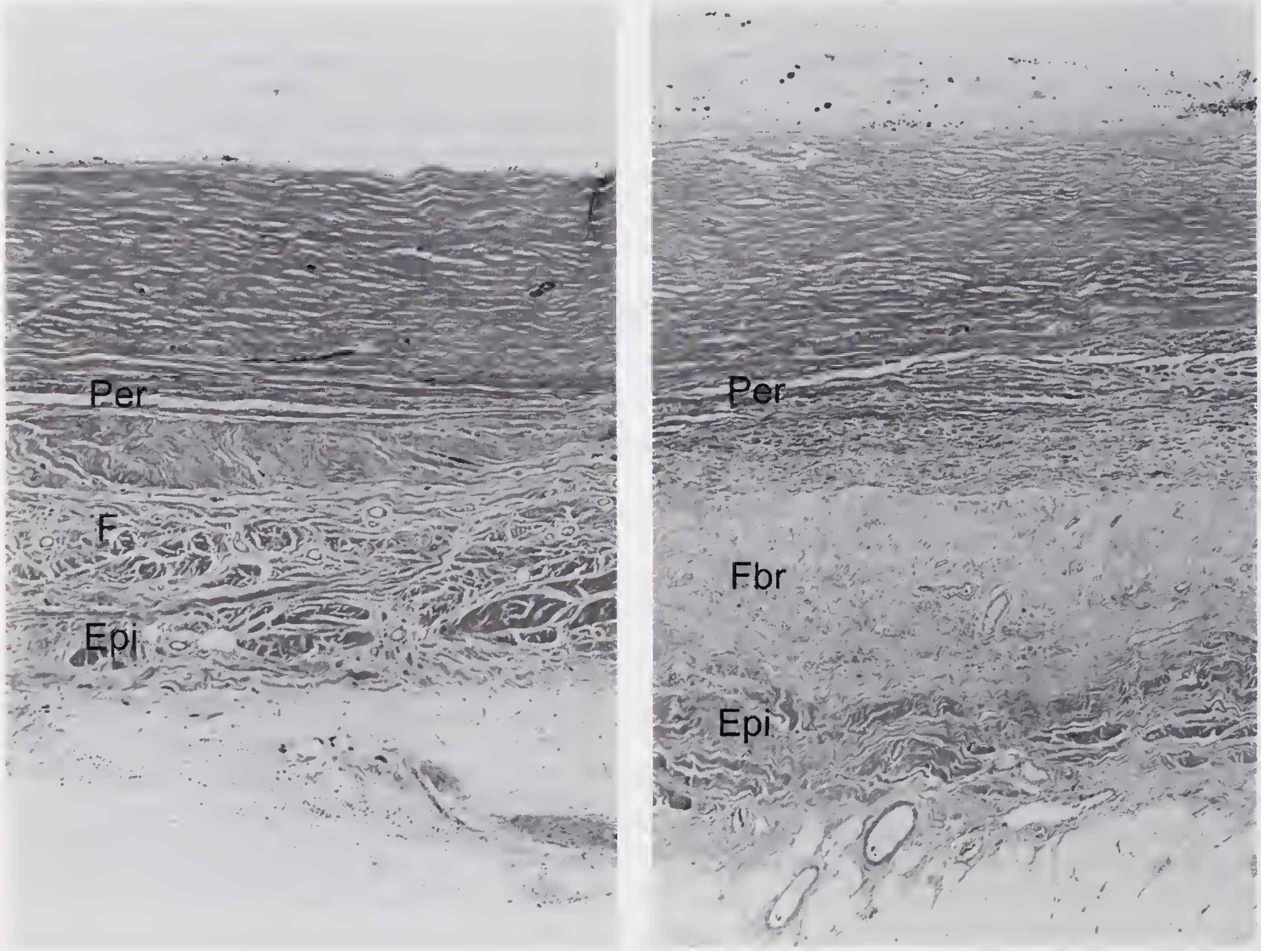

Fig. 4. Fibrous pericarditis. (A): Parietal pericardial (Per) and epicardial (Epi) layers showing thickening, marked sclerosis, and fusion (F) of both layers. (B): In some areas of the fused pericardium, focal fibrovascular proliferation (Fbr) is seen with loose cellular stroma and paucity of mature collagen, suggesting an ongoing repair process. (Hematoxylin and eosin stain and magnification 40x).

cific cause, as in this case, there may have been a prior viral pericarditis that was relatively mild or subclinical (13).

Final Pathologic Diagnosis:

Fibrous pericarditis of unknown etiology, consistent with the clinical diagnosis of constrictive pericarditis.

Clinical Follow-up:

The patient tolerated the pericardiectomy without complications. One month after this procedure, the patient was readmitted for emergency repair of a ruptured umbilical hernia. The patient tolerated this surgery without any cardiac complications. Over the subsequent months, the patient had no evidence of heart failure. Diuretics and digoxin had been discontinued. The patient recently has become morbidly obese and at the last office visit weighed 306 pounds. He has sustained vertebral lumbar compression fractures and his diabetes has become more difficult to control.

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Value of Assessing Cryptococcal Antigen in Bronchoalveolar Lavage and Sputum Specimens from Patients with AIDS

Edward J. Bottone, Ph.D., Monica Sindone, B.S., and Victor Caraballo

Abstract

Cryptococcus neoformans has become a significant opportunistic pathogen, accounting for 8 – 10% of infectious complications in patients with AIDS. When encapsulated yeast cells are observed in Giemsa-stained smears of bronchoalveolar washings (BAL), or induced sputum specimens, confirmation as *C. neoformans* is germane to definitive therapy. We therefore studied 30 BAL and 9 induced sputum specimens for cryptococcal antigen. Of the 30 BAL, 3 specimens were positive for cryptococcal antigen, ranging in titer from 1:4 to 1:256, and 2 of 9 sputum samples were also smear, culture and antigen positive (titer 1:2) for *C. neoformans*. Of the 34 negative specimens, none of the seven containing *Candida* species or the one containing *H. capsulatum* or the one containing *P. carinii* cross-reacted with cryptococcal anticapsular antibody. Our results indicate that when yeast forms suggestive of *C. neoformans* are visualized on direct smears of BAL or sputum samples, rapid confirmation as *C. neoformans* may be achieved by assessment for cryptococcal antigen. A correlation may also exist between antigen titer in respiratory specimens and extent of cryptococcal infection. **Key Words:** Cryptococcal antigen, bronchoalveolar lavage, AIDS.

Introduction

CRYPTOCOCCUS NEOFORMANS, an encapsulated yeast, has emerged as a significant opportunistic pathogen in patients with AIDS, accounting for 8-10% of their infectious complications (1, 2). While cryptococcal meningitis is most often the presenting complication, primary pulmonary involvement may be the initial stage in the pathogenesis of central nervous system disease (1, 2).

In the setting of AIDS, several opportunistic pathogens, including *C. neoformans*, may produce diffuse pulmonary involvement with clinical and radiologic findings indistinguishable from those produced by *Pneumocystis carinii* (3). When encapsulated yeast forms are seen on direct smears of bronchoalveolar lavage (BAL) and induced sputum specimens, rapid confirmation as *C. neoformans* is germane to definitive treatment. We therefore assessed the value of detecting and titrating cryptococcal capsular polysaccharide antigen in such respiratory specimens as a means of confirming the diagnosis of cryptococcal pulmonary infection and gauging the extent of cryptococcal infection.

Materials and Methods

Thirty BAL and 9 induced sputum samples were submitted for microbiologic analysis. In the

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From the Department of Medicine, Division of Infectious Diseases, Mount Sinai School of Medicine, New York, NY

Address correspondence to Edward J. Bottone, Ph.D., Department of Medicine, Division of Infectious Diseases, Box 1090, Mount Sinai School of Medicine, 1 East 100th Street, New York, NY 10029.

laboratory, an aliquot of the BAL specimen was removed and cytocentrifuged at 2000xG onto a slide. These preparations were then Giemsa-stained (HemaColor®, EM Diagnostics Systems, Gibbstown, NJ) and examined microscopically. Induced sputa were treated with a mucolytic agent (Mucolyse®, ProLab Diagnostics, Ontario, Canada) prior to cytocentrifugation and processing as for BAL specimens. In both instances, cryptococcal antigen detection was performed by reacting filtered supernates of these specimens with latex particles coated with anticapsular cryptococcal antibody (Calas®, Meridan Diagnostics, Norwich, OH) to an endpoint titration. Titers were determined according to the manufacturer's protocol, and the highest dilution rendering clear-cut agglutination was considered positive. Positive and negative cryptococcal controls were included with each test. Controls for specificity of cryptococcal antibody consisted of growing *C. neoformans* and several *Candida* species individually in brain heart infusion broth for 72 hours and reacting culture filtrates as above. Specimens obtained directly from patients were cultured for bacterial and fungal micoorganisms.

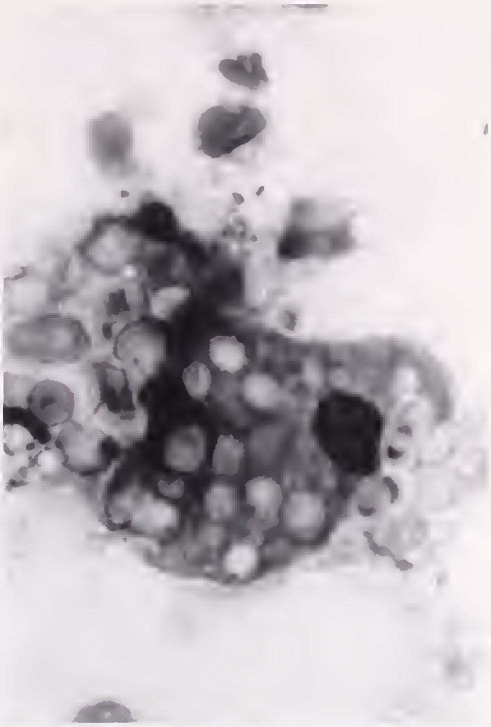

Fig. Giemsa stain of bronchoalveolar lavage showing a cluster of encapsulated yeast cells of *Cryptococcus neoformans*.

Results

Three of the 30 BAL specimens showed the presence of oval yeast cells with varying degrees of encapsulation which were morphologically suggestive of *C. neoformans* (Fig.). Oval yeast forms were also observed within pulmonary macrophages; these morphologically resembled Histoplasma capsulatum. Interaction of the 3 filtered supernates with anticapsular antibody resulted in endpoint titrations ranging from 1:4 to 1:256 (Table). *C. neoformans* only was subsequently recovered from culture of these specimens. Among the 27 negative BAL specimens, 1 showed *P. carinii*, 3 grew *Candida albicans*, 1 *Candida glabrata*, 1 *Candida* species, and 1 *H. capsulatum* (Table).

Two of the 9 sputum samples tested, reacted with anticryptococcal antibody to a titer of 1:2. Of the 7 negative specimens, 2 grew *C. albicans*. Taken together, of 39 respiratory specimens tested, 5 were smear, culture, and antigen positive for *C. neoformans*. None of the 9 specimens containing other mycotic agents or *P. carinii* cross-reacted with cryptococcal anticapsular antibody. Furthermore, 72-hour-old filtrates of 1 strain each of *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. glabrata* grown in brain heart infusion broth

TABLE

Results of cryptococcal antigen detection in respiratory specimens showing encapsulated yeast cells on smear and C. neoformans on culture

	Tested (n)	Positive (titer) (n)	Negative (n)	Species present in negative specimens (n)
Bronchoalveolar Lavage	30	3 (1:4, 1:4, 1:256)	27	*P. carinii* (1), *C. albicans* (3) *C. glabrata* (1), *Candida* spp:(1) *H. capsulatum* (1)
Sputum	9	2 (1:2, 1:2)	7	*C. albicans* (2)
Total	39	5	34	*Candida spp*:(7) *H. capsulatum* (1) *P. carinii* (1)

n = number

were non-reactive in the latex agglutination test while *C. neoformans* grown similarly was strongly reactive.

Discussion

The pathogenesis of cryptococcosis begins by inhalation of sparsely or non-encapsulated particles of *C. neoformans* from contaminated environments, usually soil (4). Despite this route of acquisition, clinically apparent pulmonary disease is a rare occurrence among immunologically competent individuals (5). In the setting of immunosuppression, however, cryptococcal infection may target the lung, or concomitantly disseminate hematogenously to other organs (1–3, 5, 6). In patients with AIDS, cryptococcal pneumonia may radiologically mimic *Pneumocystis* pneumonia or even coexist with *P. carinii* infection (1, 6–8). Because of the difficulty in making a specific etiologic diagnosis of pulmonary infiltrates in patients with AIDS, specimens obtained by bronchoscopy or sputum induction are often submitted for microbiologic evaluation. When yeast cells morphologically compatible with *C. neoformans* are visualized in Giemsa-stained smears of respiratory specimens, a tentative diagnosis of pulmonary cryptococcosis can be made. Strong support for this diagnosis can be readily achieved through demonstrating cryptococcal antigen in such respiratory specimens.

The pulmonary phase of cryptococcosis may resolve spontaneously prior to dissemination or coexist with disseminated disease (1, 3, 5). In this regard, a correlation may exist between the titer of cryptococcal antigen present in respiratory specimens and the degree of pulmonary involvement and/or dissemination. For instance, in the case previously reported (3), the patient whose bronchoalveolar specimen showed a cryptococcal antigen titer of 1:256 showed widespread cryptococcal disease involving numerous organs and the meninges. Cerebrospinal fluid cryptococcal antigen titer was 1:8192. This patient expired within 48 hours of hospital admission. Baughman et al. (9) and Meyohas et al. (6) also found a correlation between the height of the BAL cryptococcal antigen titer and extent of extrapulmonary involvement.

Three of the 5 patients whose smears and cultures were positive for *C. neoformans* showed cryptococcal antigen titers of 1:2 to 1:4 in their respiratory specimens. Two of these patients with titers of 1:2 had no evidence of disseminated disease and their serum antigen titers were negative. The third patient (titer 1:4) had been treated for cryptococcal pneumonia and meningitis that had been diagnosed earlier. The presence of both cryptococci on smear with concomitant antigen may reflect a persistent pulmonary focus of cryptococcosis or reactivation of the earlier disease, necessitating bronchoscopy as a diagnostic endeavor. This patient's concurrent serum antigen titer was 1:2.

Baughman et al. (9), in their study of the diagnostic utility of detecting cryptoccal antigen in BAL fluid, encountered 3 patients with titers of 1:2-1:4 whose smears were negative, but whose cultures of the BAL fluid were positive for *C. neoformans*. None of these patients had serum antigenemia, suggesting an early pulmonary focus without dissemination. Similarly, in their study of 27 patients with pulmonary cryptococcosis, Meyohas et al. (6) diagnosed 2 patients only by detection of cryptococcal antigen (titers not given) in BAL fluid. These patients were subsequently shown to have systemic cryptococcosis. In this report, we have shown that a diagnosis of pulmonary cryptococcosis may be readily confirmed by demonstrating cryptococcal antigen in respiratory specimens showing yeast cells morphologically resembling *C. neoformans*. Furthermore, although limited data exist from this and previous reports (6, 9), there does appear to be a correlation between antigen titer in respiratory specimens and extent of cryptococcal infection. Because cross-reactions did not occur in the presence of other mycotic agents which may be confused with cryptococci in direct smears, there was a high degree of both sensitivity and specificity accorded to the detection of cryptococcal antigen in respiratory specimens, especially if antigen titers of ≥ 1:8 are obtained (9). Although we did not correlate the presence of cryptococcal antigen with CD4$^+$ lymphocyte count, Meyohas et al. (6) recommend that cryptococcal antigen be assayed in all swab samples of BAL and pleural samples of HIV-infected patients with CD4$^+$ lymphocyte counts of < 100/mm^3. A positive reaction may signal the need for a more extensive evaluation of the patient(s) for cryptococcal infection.

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Pyoderma Gangrenosum

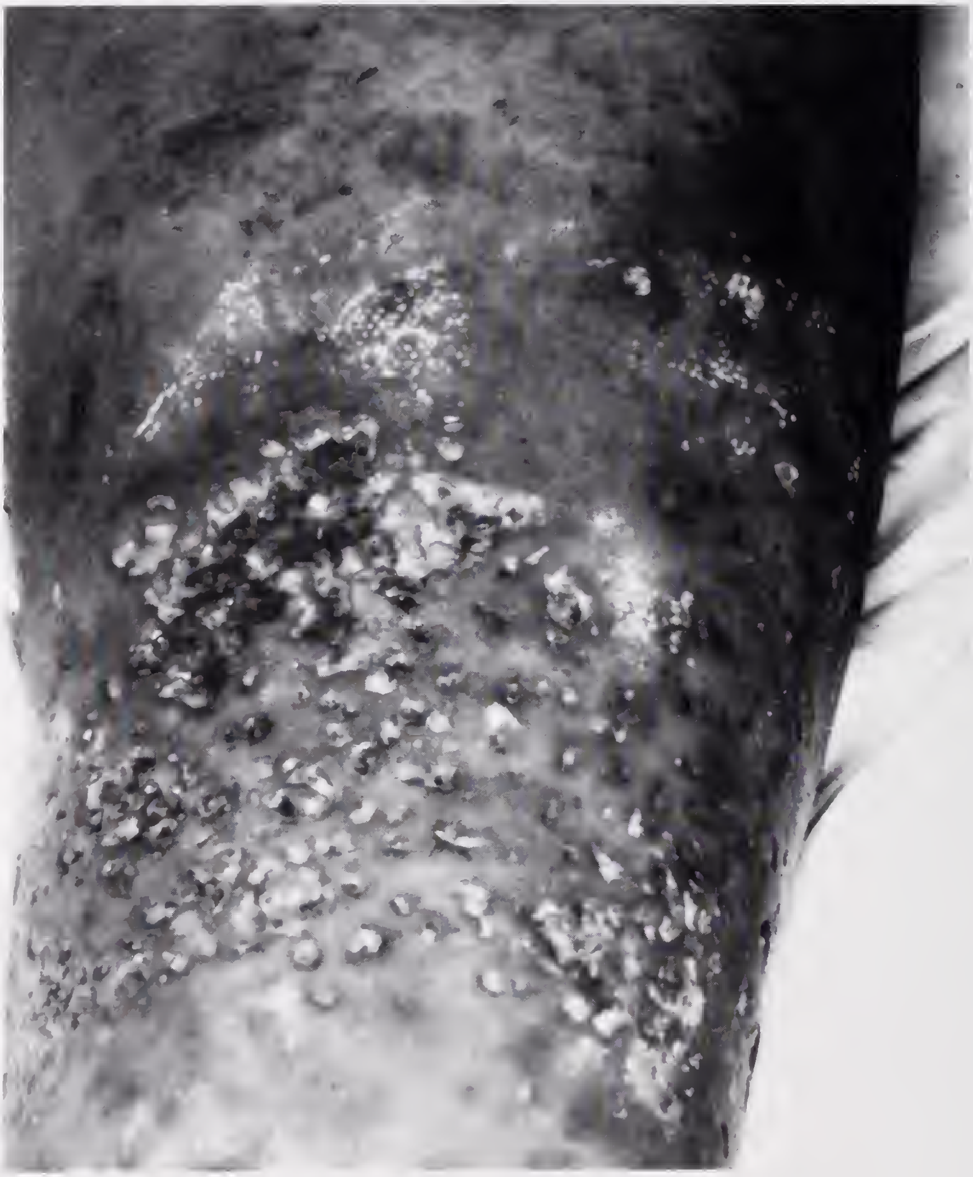

Fig. 1. Leg ulcers with crater-like holes are a characteristic manifestation of pyoderma gangrenosum. This condition is prominently associated with Crohn's disease and with ulcerative colitis, but can result from a number of other disorders. —*Michael Fellner, M.D., and Mark Lebwohl, M.D.*

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David C. Wolf

AUTHOR, SUBJECT, TITLE INDEX to VOLUME 65

A. actinomycetemcomitans: 65(5):362–369
Abe H: see Kohshi K
Abortion: 65(3):178–174
Abstracts, Research in Medicine 1997, Mount Sinai School of Medicine: 65(1):43–80
ACE inhibitor: 65(2):108–117
Acquired immunodeficiency syndrome: 65(1):22–26, 33–39; 65(6):422–425
Adhesion: 65(4):246–256
Adhesion molecules: 65(3):224–231
African Americans: 65(2):139–145
Aged: 65(4):237–245
AIDS: see acquired immunodeficiency syndrome
Alberto PL, Implant reconstruction of the jaws and craniofacial skeleton: 65(5):316–321
Aldosterone: 65(2):81–86, 87–96
alpha-fetoprotein screening: 65(3):173–177
Amaranthin lectin binding in the rat colon: response to dietary manipulation, EO Atillasoy, A Kapetanaris, SH Itzkowitz, PR Holt: 65(2):146–153
Amniocentesis: 65(3):173–177
Analgesia, postoperative: 65(4):271–279
Anemia: 65(5):309–315
Anesthesia for nonobstetric surgery during pregnancy, Y Beilin: 65(4):265–270
Angiotensin: 65(2):81–86; 87–96; 108–117
Angiotensin II and cardiac remodeling, WA Hseuh, YS Do, R Jeyaseelan: 65(2):104–107
Angiotensin, receptor: 65(2):108–117Angiotensin-converting enzyme: 65(2):81–86, 87–96
Angiotensin-converting enzyme, inhibition: 65(2):104–107, 108–117
Angiotensinogen: 65(2):81–86
Antagonizing excitotoxicity: a therapeutic strategy for stroke? D Choi: 65(2):133–138
Antibiotic prophylaxis in dentistry, A Montazem: 65(5):388–391
Antibodies, HIV: 65(1):22–26
Antidiuretic hormone, inappropriate secretion: 65(4):284–288
Apoptosis: 65(2):133–138; 65(4):246–256
Assisted suicide: 65(4):257–264
Asthma: 65(4):246–256
Atillasoy EO, Kapetanaris A, Itzkowitz SH, Holt PR, Amaranthin lectin binding in the rat colon: response to dietary manipulation: 65(2):146–153
Atlas SA, Renin-angiotensin system revisited: classical and non-classical pathways of angiotensin formation: 65(2):87–96
Atlas SA, Rosendorff C, Introduction: the renin-angiotensin system-from Tigerstedt to Goldblatt to ACE inhibition and beyond: 65(2):81–86
Atlas SA: see Rosendorff C
Autocrine: 65(2):87–96
Autocrine-paracrine factors: 65(2):118–124
AZT: 65(1):5–13

Banks K, Merlino PG, Minor oral injuries in children: 65(5):333–342
Barasch A, Safford M, Eisenberg E, Oral cancer and oral effects of anticancer therapy: 65(5):370–377
Barr DR: see Gerson MJ
Bastillo M: 65(3):215–223
Baumrin S: 65(3):215–223
Beilin Y, Anesthesia for nonobstetric surgery during pregnancy: 65(4):265–270
Benkov, KJ: see Peters VB
Bennett AJ: see Berger JT
Berger JT, Rosner F, Bennett AJ, Current events and bioethical concerns in physician-assisted death: 65(4):257–264
Berger M: see Child J
Berkowitz RL, Ethical issues involving multifetal pregnancies: 65(3):185–190
Berkowitz RL: 65(3):215–223
Bioethics: 65(4):257–264
Birnbaum AH: see Peters VB
Blam ME, editor, Clinicopathological conference: The Mount Sinai Hospital: 65(6):414–421
Bluestone BP, How perfect? A case of prenatal diagnosis: 65(3):213–214
Bluestone BP: 65(3):215–223
Bochner BS: see Grayson MH
Bottone EJ, Sindone M, Caraballo V, Value of assessing cryptococcal antigen in bronchoalveolar lavage and sputum specimens for patients with AIDS: 65(6):422–425
BRCA1: 65(3):178–174
Brensilver JM, Smith L, Lyttle CS, Impact of the Libby Zion case on graduate medical education in internal medicine: 65(4):296–300
Bronchoalveolar lavage: 65(6):422–425
Burning mouth syndrome, SA Miyamoto, VB Ziccardi: 65(5):343–347

Cancer: 65(5):370–377
Caraballo V; see Bottone EJ
Cardiac remodeling: 65(2):104–107
Cardiac tamponade in a patient with AIDS: a review of pericardial diseases in patients with HIV infection, L Estok, F Wallach: 65(1):33–39
Case reports, 65(4):280–283; 65(4):284–288; 65(4):288–291; 65(4):292–295
Cases and doubts, RE Zinberg, 65(3):210–212
Cassel CK, In sickness and in health: 65(2):154–157; Money, medicine and Methuselah: 65(4):237–245
Cathepsins: 65(2):87–96
Changing moral landscape of human reproduction: two moments in the history of *in vitro* fertilization: 65(3):167–172
Chemokines: 65(4):246–256
Chemotherapy: 65(5):370–377
Cheung T: see Weinberg RS
Child J, Berger M, Eagle K, Unexpected factors predict control of hypertension in a hospital-based homeless clinic: 65(4):304–307
Choi D, Antagonizing excitotoxicity: a therapeutic strategy for stroke? 65(2):133–138
Chorionic villus sampling: 65(3):173–177
Chusid ED: see Weinberg RS
Chymase: 65(2):87–96
Clinicopathological conference: The Mount Sinai Hospital, ME Blam, editor, N Mann, M Kim, D Purohit: 65(6):414–421
Colon: 65(2):146–153
Colp Award: 65(3):234
Commencement address, Mount Sinai School of Medicine, May 16, 1997, CK Cassel: 65(3):154–157

Compliance: 65(4):304–307

Complications of subcutaneous injection ports, FA Dysarz III, MA Fiorillo, PG Davidson: 65(4):289–291

Concepts of preemptive analgesia for postoperative pain, MR Wassef: 65(4):271–279

Cooke KJ, Human fertility and differential birth rates in American eugenics and genetics: a brief history: 65(3):161–166

Crohn's disease: 65(5)309–315; 65(6):398–403

Cryptococcal antigen: 65(6):422–425

Current events and bioethical concerns in physician-assisted death, JT Berger, F Rosner, AJ Bennett: 65(4):257–264

Cystic fibrosis: 65(3):178–184

Cytokines: 65(4):246–256

D4T: 65(1):5–13

DDI: 65(1):5–13

David RA, Rhee M, Impact of language as a barrier to effective health care in an underserved urban Hispanic community: 65(6):393–397

Davidson PG: see Dysarz III FA

Deans' Seminar: Antagonizing excitotoxicity: a therapeutic strategy for stroke? 65(2):133–138

Decompression sickness: 65(4):280–283

Dental alveolar trauma: 65(5):333–342

Dental implant: 65(5):316–321

Dermatology Notes:
  Generalized Morphea: 65(4):308; Pyoderma Gangrenosum: 65(6):422; Reactive perforating collagenosis: 65(2):158; Risks of HIV infection: 65(1):40

Diagnosis and management of tuberculosis in patients with HIV infection, DN Rose, S Vetter, A Gurtman: 65(1):14–21

Diagnosis, fetal: 65(3):173–177

Diagnosis of oral ulcers, LC Schneider, AE Schneider: 65(5):383–387

Diagnosis, prenatal: 65(3):173–177

Diamond JA: see Phillips RA

Differential diagnosis of orofacial pain, RA Pertes 65(5):348–354

Diseases, hereditary: 65(3):161–166

Divers, Japanese: 65(4):280–283

Diving: 65(4):280–283

Diving, breath-hold: 65(4):280–283

Do YS: see Hseuh WA

Doctor-patient relationship: 65(6):393–397

Dons K: see Levi N

Doyle JL: see Long RG

Dysarz III FA, Fiorillo MA, Davidson PG, Complications of subcutaneous injection ports: 65(4):289–291

Dysbaric accident: 65(4):280–283

Dysgeusia: 65(5):342–347

Dysphagia: 65(5):342–347

Eagle K: see Child J

Editorial, medical editors trial accounting (META): 65(1):41–42; The Libby Zion case revisited: what have we learned? 65(4):301–303

Editor's Note: Clinicopathologic conferences: 65(6):i

Effect of antiviral drugs and hematopoietic growth factors on *in vitro* erythropoeisis, RS Weinberg, ED Chusid, Y Galperin, JAC Thomson, T Cheung, HS Sacks: 65(1):5–13

Eisenberg E: see Barasch A

Elderly, frail: 65(4):237–245

Elizalde-Sciavolino C, Racco A, Proscia-Lieto T, Kleiner M, Severe hyponatremia, neuroleptic malignant syndrome, rhabdomyolysis and acute renal failure: a case report: 65(4):284–288

Elmer's Glue, Elsie and you: clinical application of adhesion molecules, LM Schnapp: 65(3):224–231

End of motherhood as we have known it, PS Mann: 65(3):195–200

End-stage renal disease: 65(1):27–32

Endocarditis, infective: 65(5):388–391

Eosinophil: 65(4):246–256

Equivocal notions of accuracy and genetic screening of the general population, KC Smith: 65(3):178–184

Erythropoiesis: 65(1):5–13

Estok L, Wallach F, Cardiac tamponade in a patient with AIDS: a review of pericardial diseases in patients with HIV infection: 65(1):33–39

Ethical issues involving multifetal pregnancies, RL Berkowitz: 65(3):185–190

Ethics, medical: 65(3):167–172, 215–223

Eugenics: 65(3):161–166

Euthanasia: 65(4):257–264

Experimentation, human: 65(3):167–172

Families: 65(3):195–200

Fellner M, Lebwohl M, Dermatology Notes: Pyoderma Gangrenosum, 65:(6)426

Feminism: 65(3):201–209

Fenesy KE, Periodontal disease: an overview for physicians: 65(5):362–369

Fetal blood sampling: 65(3):173–177

Fetal diagnosis: 65(3):173–177

Fibronectin: 65(2):104–107

Fiorillo MA: see Dysarz III FA

Friedman Jr F: see Kalir T

Galperin Y: see Weinberg RS

Gaucher Disease: 65(3):210–212

Generalized Morphea, M Gordon, M Lebwohl: 65(4):308

Genetic counseling: 65(3):173–177

Genetic determinism: 65(3):178–174

Genetic screening: 65(3):173–177, 178–184; 65(6):406–413

Genetic therapy: 65(6):406–413

Genetics, history of: 65(3):161–166

Geriatrics: 65(4):237–245

Gerson MJ, Schonholtz J, Grega CH, Barr DR, Importance of the family context in inflammatory bowel disease: 65(6):398–403

Gingival ulcers: 65(5):383–387

Gharavi A: see Phillips RA

Gharavi A, Klotman P, Renin-angiotensin system as a m ediator of renal injury in hypertension: 65(2):118–124

Globus Award: 65(3):234

Glomerulosclerosis: 65(1):27–32

Glossodynia: 65(5):342–347

Glutamate: 65(2):133–138

GME: 65(4):296–300

Gordon M, Lebwohl M, Generalized morphea: 65(4):308

Graduate Medical Education: 65(4):296–300

Grand Rounds, Elmer's Glue, Elsie and you: clinical application of adhesion molecules: 65(3):224–231; Money, medicine and Methuselah: 65(4):237–245; New concepts in the pathogenesis and treatment of allergic asthma: 65(4):246–256

Grayson MH, Bochner BS, New concepts in the pathogenesis and treatment of allergic asthma: 65(4):246–256
Grega CH: see Gerson MJ
Growth failure as the first expression of malnutrition in children with human immunodeficiency virus infection, VB Peters, JL Rosh, L Mugrditchian, AH Birnbaum, KL Benkov, DS Hodes, NS LeLeiko: 65(1):1–4
Gurtman A: see Rose DN
Gynandroblastoma in pregnancy: case report and review of literature, T Kalir, F Friedman Jr: 65(4):292–296

Health policy: 65(4):237–245
Heart failure: 65(6):414–421
Heir J, Ziccardi VB, Transmission of infectious disease in the dental setting: 65(5):378–382
Hemin: 65(1):5–13
Hereditary diseases: 65(3):161–166
Hepatitis: 65(5):378–382
Hirschhorn K, Reproductive choice and public policy: 65(3):191–194
Hirschhorn K: 65(3):215–223
Hispanics: 65(2):139–145; 65(6):393–397
History of human reproduction: 65(3):167–172
History of medicine: 65(3):161–166
HIV: 65(1):22–26, 33–39, 40; 65(5):322–332, 378–382
HIV-Associated nephropathy, J Winston , PE Klotman: 65(1):27–32
Hlousek L: see Long RG
Hodes DS : see Peters VB
Holt PR: see Atillasoy EO
Homeless persons: 65(4):304–307
How perfect? A case of prenatal diagnosis, BP Bluestone: 65(3):213–214
Hseuh WA, Do YS, Jeyaseelan R, Angiotensin II and cardiac remodeling: 65(2):104–107
Human fertility and differential birth rates in American eugenics and genetics: a brief history, KJ Cooke: 65(3):161–166
Human immunodeficiency virus: 65(1):1–4, 33–39
Human immunodeficiency virus - AIDS, theme issue, guest editor, H. Sacks: 65(1):1–39
Huton J: see Lewis M
Hypertension, treatment of: 65(2):87–96
Hypertension: 65(2):81–86, 108–117, 125–132; 65(4):304–307
Hypertrophy, vascular: 65(2):108–117
Hyponatremia: 65(4):284–288
Hypoxia: 65(2):133–138

Ideology and technology: The social context of procreative technology, BK Rothman: 65(3):201–202
IgE: 65(4):246–256
IgG subclasses: 65(2):139–145
Immerman MM, Personal reflection on a time past: 65(3):232–233
Immunization, passive: 65(1):22–26
Immunoglobulin and IgG subclass levels in the African American and Hispanic populations of East Harlem, M Lewis, M Miyashiro, J Huton, L Miller, K Sperber: 65(2):139–145
Immunotherapy: 65(4):246–256
Impact of language as a barrier to effective health care in an underserved urban Hispanic community, RA David, M Rhee: 65(6):393–397
Impact of the Libby Zion case on graduate medical education in internal medicine, JM Brensilver, L Smith, CS Lyttle: 65(4):296–300
Implant reconstruction of the jaws and craniofacial skeleton, PL Alberto: 65(5):316–321
Importance of the family context in inflammatory bowel disease, MJ Gerson, J Schonholtz, CH Grega, DR Barr: 65(6):398–403
In sickness and in health, CK Cassel: 65(2):154–157
In vitro fertilization: 65(3):167–172
Infarction, cerebral: 65(4):280–283
Inflammatory bowel disease: 65(6):398–403
Infuse-a-Port®: 65(4):289–291
Injection ports, subcutaneous: 65(4):289–291
Integrins: 65(3):224–231
Introduction: the renin-angiotensin system-from Tigerstedt to Goldblatt to ACE inhibition and beyond, SA Atlas, C Rosendorff: 65(2):81–86
Ischemia: 65(2):133–138
Issues in medical ethics, panel discussion, R Rhodes, DA Moros: 65(3):215–223
Itzkowitz SH: see Atillasoy EO

Jacobson JM, Passive immunization for the treatment of HIV infection: 65(1):22–26
Jeyaseelan R: see Hseuh WA
Judaism, genetic screening and genetic therapy, F Rosner: 65(6):406–413

Kalir T, Friedman Jr F, Gynandroblastoma in pregnancy: case report and review of literature 65(4):292–296
Kapetanaris A: see Atillasoy EO
Kim M: see Blam ME
Kinoshita Y: see Kohshi K
Kleiner M: see Elizalde-Sciavolino C
Klotman P: see Gharavi A
Kohshi K, Kinoshita Y, Abe H, Okudera T, Multiple cerebral infarction in Japanese breath-hold divers: two case reports: 65(4):280–283
Krakoff LR, Rajasekharaiah V, Preventing and reversing target organ damage by treatment of the renin-angiotensin system: 65(2):125–132
Kupfer S, *Editorial*, medical editors trial accounting (META): 65(1):41–42; Editor's note: 65(5 & 6):i
Kurtin S: see Lebwohl M

Lebwohl M, Kurtin S, Reactive perforating collagenosis: 65(2):158
Lebwohl M: see Gordon M; Shelton R; see Fellner M
Lectin, binding: 65(2):146–153
Left ventricular hypertrophy, reversal of: 65(2):125–132
Left ventricular hypertrophy in hypertension: etiology, treatment, and controversies, RA Phillips, JA Diamond, A Gharavi: 65(2):87–96
LeLeiko NS: see Peters VB
Leucocyte adhesion deficiency syndrome: 65(3):224–231
Leucocyte-endothelial interactions: 65(3):224–231
Leukemia: 65(5):309–315
Levi N, Dons K, Two-level thoracic disc herniation: 65(6):404–405
Lewis M, Miyashiro M, Huton J, Miller L, Sperber K, Immunoglobulin and IgG subclass levels in the African American and Hispanic populations of East Harlem: 65(2):139–145
Libby Zion case revisited: what have we learned? B Stimmel: 65(4):301–303
Life expectancy: 65(4):237–245
Long RG, Hlousek L, Doyle JL, Oral manifestations of systemic diseases: 65(5):309–315

Longevity: 65(4):237–245
Losartan: 65(2):108–117
Lyttle CS: see Brensilver JM

Malnutrition: 65(1):1–4
Mann, N: see Blam ME
Mann PS: End of motherhood as we have known it: 65(3):195–200
Mann PS: 65(3):215–223
Manpower: 65(4):296–300
Merlino PG: see Banks K
META: 65(1):41–42
Metastasis; 65(5): 309–315
Miller L: see Lewis M
Minor oral injuries in children, K Banks, PG Merlino: 65(5):333–342
Miyamoto SA, Ziccardi VB, Burning mouth syndrome: 65(5):343–347
Miyashiro M: see Lewis M
Money, medicine and Methuselah, CK Cassel: 65(4):237–245
Montazem A, Antibiotic prophylaxis in dentistry: 65(5):388–391
Morbidity: 65(4):265–270
Moros DA: see Rhodes R
Morphea: 65(4):308
Motherhood: 65(3):195–200
Mugrditchian L: see Peters VB
Multifetal pregnancy, reduction: 65(3):185–190
Multiple cerebral infarction in Japanese breath-hold divers: two case reports, K Kohshi, YKinoshita, H Abe, T Okudera: 65(4):280–283
Mycobacterium avian-intracellulare: 65(1):33–39
Myocytes, cardiac: 65(2):104–107

Neoplasm: 65(5):309–315
Neuroleptic malignant syndrome: 65(4):284–288
Neurotoxicity: 65(2):133–138
New concepts in the pathogenesis and treatment of allergic asthma, MH Grayson, BS Bochner: 65(4):246–256
Nitric oxide: 65(2):104–107
Nutrition: 65(5):355–361

Obstetrics: 65(4):265–270
Okudera T: see Kohshi K
Oral cancer and oral effects of anticancer therapy, A Barasch, M Safford, E Eisenberg: 65(5):370–377
Oral manifestations of HIV disease, DA Sirois: 65(5):322–332
Oral manifestations of nutrient deficiencies, R Touger-Decker: 65(5):355–361
Oral manifestations of systemic diseases, RG Long, L Hlousek, JL Doyle; 65(5):309–315
Oral Medicine, theme issue, guest editor, VB Ziccardi: 65(5):309–391
Oral soft tissue injuries: 65(5):333–342
Orofacial pain: 65(5):347–354
Osseointegration: 65(5):316–321
Osteopontin: 65(2):104–107
Ovary: 65(4):292–296

P. gingivalis: 65(5):362–369
Pain, postoperative: 65(4):271–279
Panel discussion, Theme issue on reproductive choice: 65(3):215–223
Paracrine: 65(2):87–96
Passive immunization for the treatment of HIV infection, JM Jacobson: 65(1):22–26
Patients' rights: 65(4):257–264
Patriarchy: 65(3):201–209
Perforating collagenosis: 65(2):158
Pericarditis: 65(1):33–39
Pericardium: 65(6):414–421
Periodontal disease: an overview for physicians, KE Fenesy: 65(5):362–369
Periodontitis: 65(5):362–369
Personal reflection on a time past, MM Immerman: 65(3):232–233
Pertes RA, Differential diagnosis of orofacial pain: 65(5):348–354
Peters VB, Rosh JR, Mugrditchian L, Birnbaum AH, Benkov KJ, Hodes DS, LeLeiko NS, Growth failure as the first expression of malnutrition in children with human immunodeficiency virus infection: 65(1):1–4
Phillips RA, Diamond JA, Gharavi A, Left ventricular hypertrophy in hypertension: etiology, treatment, and controversies: 65(2):87–96
Polydipsia, psychogenic: 65(4):284–288
Port-a-cath®: 65(4):289–291
Pregnancy: 65(4):265–270; 292–296
Prenatal care: 65(3):201–209
Prenatal diagnosis: 65(3):173–177
Prenatal testing: 65(3):201–209
Preventing and reversing target organ damage by treatment of the renin-angiotensin system, LR Krakoff, V Rajasekharaiah: 65(2):125–132
Prolapse: 65(6):404–405
Proliferative markers: 65(2):146–153
Proscia-Lieto T: see Elizalde-Sciavolino C
Public policy: 65(3):191–194
Purohit DP: see Blam ME
Pyoderma gangrenosum: 65(6):422

Racco A: see Elizalde-Sciavolino C
Radiation therapy: 65(5):370–377
Rajasekharaiah V: see Krakoff L
Reactive perorating collagenosis, M Lebwohl, S Kurtin: 65(2):158
Refeeding: 65(2):146–153
Renal disease, end-stage: 65(2):118–124
Renal failure: 65(1):27–32; 65(4):284–288
Renal injury, nonhemodynamic mechanisms: 65(2):118–124
Renin: 65(2):81–86, 87–96, 108–117
Renin-angiotensin system: 65(2):125–132
Renin-angiotensin system and target organ damage in hypertension, theme issue, guest editors, C Rosendorff, SA Atlas: 65(2):81–132
Renin-angiotensin system as a mediator of renal injury in hypertension, A Gharavi, P Klotman:65(2):118–124
Renin-angiotensin system revisited: classical and nonclassical pathways of angiotensin formation, SA Atlas: 65(2):87–96
Reproductive choice and public policy, K Hirschhorn: 65(3):191–194
Reproductive choice: genetic counseling and assisted reproductive technology, theme issue, guest editors, R Rhodes, DA Moros, A Robichaud: 65(3):159–223
Reproductive genetics and today's patient options: prenatal diagnosis, JP Willner: 65(3):173–177
Reproductive technologies: 65(3):178–174, 195–200, 201–209
Research in Medicine, 1997, Mount Sinai School of Medicine: 65(1):43–80

Rhabdomyolysis: 65(4):284–288
Rhee M: see David RA
Rheumatology: 65(5):309–315
Rhodes R, Moros DA, Issues in medical ethics, panel discussion: 65(3):215–223
Rhodes R, Moros DA, Robichaud A, guest editors, theme issue, Reproductive choice; genetic counseling and assisted reproductive technology: 65(3):159–223
Rhodes R, Moros DA, Robichaud A, Introduction to theme issue: 65(3):159
Risks of HIV infection, Shelton R, Lebwohl M: 65(1):40
Robichaud A: see Rhodes R
Rose DN, Vetter S, Gurtman A, Diagnosis and management of tuberculosis in patients with HIV infection: 65(1):14–21
Rosendorff C: see Atlas SA
Rosendorff C, Vascular hypertrophy in hypertension: role of the renin-angiotensin system: 65(2):108–117
Rosendorff C, Atlas SA, guest editors, theme issue, Renin-angiotensin system and target organ damage in hypertension: 65(2):81–132
Rosh, JR: see Peters VB
Rosner, F, Judaism, genetic screening and genetic therapy, 65(6):406–413
Rothman BK: 65(3):215–223
Rothman BK, Ideology and technology: The social context of procreative technology: 65(3):201–202
Rosner F: see Berger JT

Sacks HS, guest editor, theme issue, Human immunodeficiency virus-AIDS: 65(1):1–39
Sacks HS: see Weinberg RS
Safford M: see Barasch A
Schnapp LM, Elmer's Glue, Elsie and you: clinical application of adhesion molecules: 65(3):224–231
Schneider AE: see Schneider LC
Schneider LC, Schneider AE, Diagnosis of oral ulcers: 65(5):383–387
Schonholtz J: see Gerson MJ
Screening, genetic: 65(3):173–177, 178–184
Selective termination: 65(3):185–190
Severe hyponatremia, neuroleptic malignant syndrome, rhabdomyolysis and acute renal failure: a case report, C Elizalde-Sciavolino, A Racco, T Proscia-Lieto, M Kleiner: 65(4):284–288
Shelton R, Lebwohl M, Risks of HIV infection: 65(1):40
Sindone M: see Bottone EJ
Sirois DA, Oral manifestations of HIV disease: 65(5):322–332
Smith KC: 65(3):215–223
Smith KC, Equivocal notions of accuracy and genetic screening of the general population: 65(3):178–184
Smith L: see Brensilver JM
Sodium balance: 65(2):81–86
Sperber K: see Lewis M
Starvation: 65(2):146–153
Stem cell factor: 65(1):5–13
Stimmel B, Libby Zion case revisited: what have we learned? 65(4):301–303
Student Corner: Unexpected factors predict control of hypertension in a hospital-based homeless clinic, J Child, M Berger, K Eagle: 65(4):304–307

Target organ, damage to: 65(2):125–132
Tay-Sachs Disease: 65(3):210–212
Testing, genetic: 65(3):173–177, 178–184
Theme Issues, Human immunodeficiency virus - AIDS: 65(1):1–39; Oral Medicine: 65(5):309–391; The renin-angiotensin system and target organ damage in hypertension: 65(2):81–132; Reproductive choice, genetic counseling, and assisted reproductive technology: 65(3):159–223
Thomson JAC: see Weinberg RS
Touger-Decker R, Oral manifestations of nutrient deficiencies: 65(5):355–361
Training: 65(4):296–300
Transmission of infectious disease in the dental setting, J Heir, VB Ziccardi: 65(5):378–382
Transpedicular surgery: 65(6):404–405
Tuberculosis: 65(1):14–21; 65(5):378–382
Two-level thoracic disc herniation, N Levi, K Dons: 65(6):404–405

Ulcerative colitis: 65(6):398–403
Ulcers, gingival: 65(5):383–387
Ulcers, mucosal: 65(5):383–387
Unexpected factors predict control of hypertension in a hospital-based homeless clinic, J Child, M Berger, K Eagle: 65(4):304–307
Uremia: 65(5):309–315

Valone DA, The changing moral landscape of human reproduction: two moments in the history of *in vitro* fertilization: 65(3):167–172, 215–223
Value of assessing cryptococcal antigen in bronchoalveolar lavage and sputum specimens for patients with AIDS, EJ Bottone, M Sindone, V Caraballo: 65(6):422–425
Vascular access: 65(4):289–291
Vascular hypertrophy in hypertension: role of the renin-angiotensin system, C Rosendorff: 65(2):108–117
Vasoconstriction: 65(2):104–107
Vesiculobullous oral lesions: 65(5):383–387
Vetter S: see Rose DN

Wallach F: see Estok L
Wassef MR, Concepts of preemptive analgesia for postoperative pain: 65(4):271–279
Weinberg RS, Chusid ED, Galperin Y, Thomson JAC, Cheung T, Sacks HS, Effect of antiviral drugs and hematopoietic growth factors on *in vitro* erythropoiesis: 65(1):5–13
Willner JP: 65(3):215–223
Willner JP, Reproductive genetics and today's patient options: prenatal diagnosis: 65(3):173–177
Winston J, Klotman PE, HIV-associated nephropathy: 65(1):27–32

Ziccardi VB, guest editor, theme issue, Oral Medicine 65(5):309–391: see Miyamoto SA; see Heir J
Zinberg RE, Cases and doubts: 65(3):210–212
Zinberg RE: 65(3):215–223

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3. Couser WG. Glomerular diseases. In: Stein J, editor. Internal medicine. 2nd ed. Boston: Little Brown; 1987. pp. 834-861.

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